GENETICS

A periodical record of investigations bearing on heredity and variation

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TABLE OF CONTENTS

New Policies for **Genetics** i

ALEXANDER, MARY L., JANET BERGENDAHL, and MADELEINE BRITTAIN, Biological damage in mature and immature germ cells of *Drosophila virilis* with ionizing radiations 979

WRIGHT, SEWALL, A quantitative study of variations in intensity of genotypes of the guinea pig at birth 1001

KRIVSHENKO, J., New evidence for the homology of the short euchromatic elements of the X and Y chromosomes of *Drosophila busckii* with the microchromosome of *Drosophila melanogaster* 1027

CLAYTON, FRANCES E., The effect of lozenge pseudoalleles on eye pigmentation in *Drosophila melanogaster*. III. Pigment development during pupal differentiation 1041

VON BORSTEL, R. C., and M. L. REKEMEYER, Radiation-induced and genetically contrived dominant lethality in Habrobracon and Drosophila 1053

GUTIERREZ, MARIO G., and G. F. SPRAGUE, Randomness of mating in isolated polycross plantings of maize 1075

FROST, HOWARD B., MARGARET M. LESLEY, and WARREN F. LOCKE, Cytogenetics of a trisomic of *Matthiola incana*, involving a ring chromosome and somatic instability of singleness (versus doubleness) of flowers and shape of leaves 1083

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1. GENETICS will be changed from a bimonthly to a monthly journal.

2. The annual volume of the journal will include a minimum of 1,000 pages. The Editors expect to include enough additional pages to print all acceptable manuscripts if funds are available.

3. The subscription rate will be $12.00 for a complete volume (January-December). Foreign postage will be $1.00 extra. Single copies will cost $1.50.

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b) A gene symbol should be defined or identified with the mutant character the first time the symbol is used in a manuscript.

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BIOLOGICAL DAMAGE IN MATURE AND IMMATURE GERM CELLS OF DROSOPHILA VIRILIS WITH IONIZING RADIATIONS[1]

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BIOLOGICAL damage resulting from radiation treatment has been studied in a number of biological systems (LEA 1947; HOLLAENDER 1954; BARQ and ALEXANDER 1955). ZIRKLE (1954) discussed the implications of the observed influence of linear energy transfer upon biological damage. More recently BURCH (1957) has discussed several problems of relative biological efficiency with respect to the methods used to characterize the quality of ionizing radiations.

In Drosophila, differences in the number of sex-linked recessive lethals recovered after X-radiation treatment of mature and immature germ cells were reported earlier by HARRIS (1929), HANSEN and HEYS (1929) and DEMEREC and KAUFMANN (1941). LUNNING (1952) reported differences in the effectiveness of X-radiation for dominant lethal damage in the spermatogenic cycle of *Drosophila melanogaster*. The characteristics of biological damage in various types of germ cells of *Drosophila virilis* subjected to fission neutrons and 14 Mev neutrons and comparisons of the results obtained with neutrons to those produced by ionizing radiations have been discussed in previous papers (ALEXANDER 1958a, b, c).

The data for translocation and dominant lethal damage produced in the spermatogenic cycle of *Drosophila virilis* by several types of ionizing radiations are reported in the present experiments. Studies have been made of relative effectiveness of high energy X-rays and γ-rays as compared with 200 kv X-rays.

MATERIAL AND METHODS

Young males of *D. virilis* were treated with 200 kv X-rays, γ-rays from cobalt-60 and 22 Mv X-rays from a 31 Mev betatron source. All treatments were in air at 27°C. With therapy X-rays, doses of 500r (X-1), 1000r (X-2) and 2000r (X-3) were given at a dose rate of 202r per minute. The machine was operated at 200 kv and 15 ma with a half value layer of 1.4 mm of copper and an effective wave length of the soft end beam of 90 kev. Conversions from roentgens to absorbed doses (rads) were made using a value of 0.96 rads per r for muscle tissue. Doses of 500r (G-4), 1000r (G-5) and 2000r (G-6) of 1.17–1.33 Mev γ-rays from a cobalt-60 source were given at a dose rate of 200r per minute. For these irradia-

[1] This investigation supported by Contract No. AT-(40-1)-1828 with the United States Atomic Energy Commission and The University of Texas, M. D. Anderson Hospital and Tumor Institute.

tion circumstances, the average absorbed dose to muscle tissue will be 0.96 rads from 1r of γ-rays.

X-rays of 22 Mv energy were obtained from a betatron source. Two different series of tests were run with one series consisting of three doses, 500r (B-4), 1000r (B-5) and 2000r (B-6). In the second series, doses of 1000 rads (B-7) and 2000 rads (B-8) were given. For the betatron treatments a four centimeter block of lucite was placed immediately above the biological material, between the material and target. A field size of ten cm was used at a distance of 85 cm between the target and biological material to obtain the maximum dose intensity from the beam. The fly chamber is, in essence, a small cavity in the lucite and the absorbed dose to the flies is therefore approximately the same as the absorbed dose to the lucite. Using the conversion unit of 0.915 rad per "nominal" r in water (SINCLAIR *et al.* 1958), the absorbed dose in rads for lucite is 0.915 ($\mu\alpha$ lucite = 0.0147, $\mu\alpha$ water = 0.0155, NBS Handbook 62, 1956[1]). Using these units, 0.87 rads results from one "nominal" roentgen unit for 22 Mv X-rays. The physical descriptions of the sources and dosimetry are included in SINCLAIR and BLACKWELL (1958).

Mature sperm tests were obtained from seven-day-old males treated with gamma rays and with 200 kv X-rays. These tests allow comparisons of the lethal damage in samples of motile, mature sperm of mature males with those for sperm bundles of young males. Treatments were made in air with dose rates of 190 or 225r per minute at 25°C. With 200 kv X-rays the tests include X-4, 500r (480 rads); X-5, 1000r (960 rads); X-6, 2000r (1920 rads). Three additional tests X-7, X-11, and X-19 were carried out with 2000r. The 3000r test is designated as X-20. The gamma ray experiments include a 500r (G-1) test, a 1000r test (G-2) and three 2000r tests (G-3, G-13, G-18).

Strain 1801.1, Texmelucan, Mexico of *Drosophila virilis* was used as the standard stock for the radiation tests. For the immature germ cell tests, adult males of the Texmelucan strain were collected at two hour intervals after eclosion, and aged 17 to 23 hours before radiation treatment. Adult males of *virilis* require six days to mature, and the first sample of treated germ cells was obtained on the sixth day after eclosion of adults from the pupae cases. The treated males were mated, individually, to three females. In all but the B-7 and B-8 tests the treated males were mated, individually, to two types of females, one "marker females" and two normal females. In experiments B-7 and B-8, the males were mated either to three marker females or to three normal females. The marker females were used for translocation tests. Marker females were homozygous for the recessive genes *b*, (broken, chromosome 2); *tb gp*, (tiny bristle and gapped, chromosome 3); *cd* (cardinal, chromosome 4); and *pe*, (peach, chromosome 5). Samples of F_1 male offspring, from crosses between treated males and marker females, were backcrossed individually to three marker females. Translocations between the second, third, fourth, fifth, and Y chromosomes were detected by genetic recombination of the F_2 offspring classes.

Dominant lethals were measured by the use of egg development tests. Normal

[1] ($\mu\alpha$ = mass of real absorption coefficient in cm^2/gm.)

heterozygous females were obtained from crosses of strain 1999 ♀ × 2375 ♂. One or two of the normal heterozygous females (used for the matings of one treated male × one marker female, two normal heterozygous females) were separated from the male and all eggs counted for each female for three or four days. Percentages of dominant lethals were calculated as the proportion of eggs which failed to develop into pupae.

The germ cells treated in young adult males were tested by mating the males, after the preliminary maturing period of six days, every two days for a period of 20 days. The first five mating periods, A–E, consisted of two day mating periods of males in all experiments. These periods represent sperm bundles and spermatid samples of postmeiotic cells. Sperm bundles are the most mature germ cells present in *virilis* males at the time of treatment—17–23 hours after eclosion. The first mating period, A, represents cells treated as sperm bundles. The second period, B, may contain mixtures of germ cells treated as sperm bundles and spermatids. Periods C, D and E are representative of germ cells treated as spermatids. The sixth mating period, F, represents cells treated in the meiotic divisions and is characterized by semisterility of the males and a low translocation rate. Sterility of the males is also obtained in the following mating period, G. The males exhibit aspermy during this period and females lay eggs which contain no sperm and therefore fail to develop (ALEXANDER and STONE 1955).

Separation of cells that were in meiotic from those in postmeiotic stages was more difficult with 22 Mv X-rays than with therapy X-rays since dominant lethal damage remained high in meiotic cells and only small samples for translocation tests were obtained in most periods. Sampling of meiotic and premeiotic cells included both two and three day mating periods. These are indicated in the tables and will be discussed in the RESULTS with the comparisons of the several types of radiations. The continuous drop in lethal damage obtained in periods H and I indicate that sampling has reached cells treated as spermatogonia. The designation of cell stages in *virilis* has been discussed more fully by ALEXANDER and STONE (1955); CLAYTON (1957) and ALEXANDER (1958a).

In the mature sperm test, seven-day-old males were treated and mated, individually, to three normal females or three marker females. Dominant lethal tests were obtained from the normal heterozygous females and translocation tests from marker females.

RESULTS

The data for the biological tests with 200 kv X-rays, γ-rays and 22 Mv X-rays are given in Tables 1, 2, and 3 and illustrated in Figures 1, 2 and 3. Data for treated mature sperm of seven-day-old males are included in Table 5. Tests with 200 kv X-ray are indicated by the letter "X" followed by the number of the test; gamma rays are represented by "G" and 22 Mv X-rays by "B." In Tables 1, 2, and 3, the experiments with the radiation doses in roentgens and the calculated relative absorbed dose expressed as rads are shown in the first column. The two-day remating periods are indicated in the second column by the letters A

TABLE 1

Translocation and dominant lethal damage with 200 kv X-ray

Experiment and dose	Period	Translocations†								Dominant lethals		
		T_2	T_3	T_{2+2}	T_4	T_{2+3}	T_5	Total sperm tested	Percent sperm with translocation	Eggs	Pupae	Percent lethals
	A	..	..	..	..	..	..	...	...	....	....	...
	B	3	0	0	0	0	0	326	0.9	3110	2944	5.3
X-1	C	..	..	..	..	..	..	...	...	1921	1738	9.5
(500r)	D	20	0	0	0	0	0	363	5.5	2279	1672	26.6
480 rads	E	16	0	0	0	0	0	235	6.8	2467	1406	43.0
Air	F*	2	0	0	0	0	0	150	1.3	2177	1700	21.9
	G*	..	..	..	..	..	..	...	...	2171	1802	17.0
	H	..	..	..	..	..	..	...	...	693	618	10.8
	A	..	..	..	..	..	..	...	...	....	....	...
	B	4	0	0	0	0	0	307	1.3	2698	2402	11.0
X-2	C	..	..	..	..	..	..	...	...	2146	1309	39.0
(1000r)	D	48	5	0	1	0	0	349	15.5	3484	1061	69.6
960 rads	E	38	12	3	4	0	0	270	21.1	2525	420	83.4
Air	F*	2	0	0	0	0	0	221	0.9	2659	915	65.6
	G*	..	..	..	..	..	..	...	...	2220	1527	31.2
	H	..	..	..	..	..	..	...	...	1451	1272	12.3
	A	..	..	..	..	..	..	...	...	....	....	...
	B	31	2	0	0	0	0	290	11.4	3578	2003	44.0
X-3	C	..	..	..	..	..	..	...	...	2555	633	75.2
(2000r)	D	47	16	6	1	0	1	174	40.8	2488	183	92.6
1920 rads	E	5	1	1	2	1	0	14	71.4	1257	35	97.2
Air	F*	1	1	0	0	0	0	13	15.4	507	16	96.8
	G*	..	..	..	..	..	..	...	...	1668	748	55.2
	H	..	..	..	..	..	..	...	...	1126	805	28.5

* Three day mating period.
† T_2, T_3, T_4, T_5, indicate translocations with the number of chromosomes involved; $T_{(2+2)}$ and $T_{(2+3)}$ indicate the recovery of two different translocations from the same cell.

through H or I. Results for 200 kv X-rays are presented in Table 1. For the biological tests, males taken at the same time were divided into three groups and each group treated with 500, 1000 or 2000r doses and the tests were run simultaneously. Data for experiments, G-4, G-5, G-6, for γ-rays from cobalt-60 are included in Table 2. The various biological tests for these experiments were run simultaneously. The betatron experiments, B-4, B-5 and B-6 (Table 3), were also made with three groups of males taken at one time and the tests for these three experiments were run at the same time. The results with the betatron radiation were so unusual that two additional tests, B-7 and B-8, were run; one dose, 1000 rads, was intermediate between B-5 and B-6, and one dose, 2000 rads, higher than B-6.

The kinds of translocations and the number recovered per cell are represented in Tables 1 and 2. Induced translocations, one per cell, involved two (T_2),

TABLE 2

Translocation and dominant lethal damage with 1.17–1.33 Mev gamma rays

Experiment and dose	Period	Translocations*								Dominant lethals		
		T_2	T_3	T_{2+2}	T_4	T_{2+3}	T_5	Total sperm tested	Percent sperm with translocation	Eggs	Pupae	Percent lethal
	A	..	..	..	..	..	..	...	...	....	....	...
	B	2	0	0	0	0	0	286	0.7	1425	1313	7.9
G-4	C	0	0	0	0	0	0	170	...	1466	1288	12.1
(500r)	D	6	0	0	0	0	0	241	2.5	905	698	22.9
480 rads	E	10	1	0	0	0	0	199	5.5	1937	962	50.3
Air	F	..	..	..	..	..	..	...	...	1311	734	44.0
	G	..	..	..	..	..	..	...	...	1573	1073	31.8
	H	..	..	..	..	..	..	...	...	....	....	...
	A	1	0	0	0	0	0	47	2.1	245	227	7.3
	B	8	0	0	0	0	0	398	2.0	2059	1752	14.9
G-5	C	3	0	0	0	0	0	100	3.0	3052	2304	24.5
(1000r)	D	14	0	1	0	0	0	224	6.7	1304	642	50.8
960 rads	E	34	3	2	1	2	0	168	25.0	2445	519	78.8
Air	F	0	0	0	0	0	0	27	...	1539	373	75.8
	G	..	..	..	..	..	..	...	...	1655	589	64.4
	H	0	0	0	0	0	0	79	...	....	....	...
	A	2	0	0	0	0	0	59	3.4	442	322	27.1
	B	25	2	1	0	0	0	309	9.1	1569	970	38.2
G-6	C	11	0	0	0	0	0	92	12.0	3050	1206	60.5
(2000r)	D	21	7	2	0	1	0	100	31.0	1930	267	86.2
1920 rads	E	9	2	0	3	0	0	28	50.0	1902	62	96.7
Air	F	2	0	0	0	0	0	4	50.0	512	14	97.3
	G	0	0	0	0	0	0	17	...	766	168	78.1
	H	..	..	..	..	..	..	...	...	....	....	...

* T_2, T_3, T_4, T_5, indicate translocations with the number of chromosomes involved; $T_{(2+2)}$ and $T_{(2+3)}$ indicate the recovery of two different translocations from the same cell.

three (T_3), four (T_4) or five (T_5) chromosomes, and two different translocations recovered from the same cell are indicated by (T_{2+2}) and (T_{2+3}). The total number of sperm in each test are given. After betatron treatment of spermatid stages, only small numbers of offspring were obtained, and since only a few translocations involved more than two chromosomes, only the total number of translocations were included (Table 3). Data for the dominant lethal tests are shown as the total number of eggs and of pupae which developed. Percentages of dominant lethals indicate the proportion of eggs which failed to develop.

Control tests for dominant lethals were carried out simultaneously with the B-7 and B-8 tests. Mature sperm from seven-day-old males and from adults, isolated as pupae, have also been tested. Results from the control crosses of 1801.1 male × normal heterozygous females (1999 ♀ × 2375.8 ♂) are included in Table 4. In the control tests, a total of 35,257 pupae developed from 38,355

TABLE 3

Translocation and dominant lethal damage with 22 Mv X-rays

Experiment and dose	Period	Translocations			Dominant lethals		
		Sample size	Number of translocations	Percent translocations	Eggs	Pupae	Percent dominant lethals
	A	333	2	0.6	1750	1620	7.4
	B	106	1	0.9	1328	1245	6.2
	C	101	1	0.9	1278	1197	6.3
B-4	D	45	0	...	1930	1769	8.3
500r	E	62	2	3.2	1303	1016	22.0
(435 rads)	F	...	..	...	1513	1219	19.4
	G	...	..	...	1198	795	33.6
	H	...	..	...	1359	911	33.0
	I	...	..	...	....	....	...
	A	356	6	1.7	1712	1510	11.8
	B	45	0	...	2492	2081	16.5
	C	127	3	2.4	2568	2073	19.3
B-5	D	116	6	5.2	2556	1657	35.2
1000r	E	57	3	5.2	1559	700	55.1
(870 rads)	F	...	..	...	2280	868	61.9
	G	...	..	...	1035	392	62.1
	H	...	..	...	720	335	53.5
	I	...	..	...	....	....	...
	A	79	0	...	335	275	22.5
	B	69	0	...	673	498	26.0
	C	121	11	9.1	1024	642	37.3
B-7	D	124	10	8.1	1358	621	54.3
1000 rads	E	...	..	...	2087	514	75.4
	F	...	..	...	1210	334	72.4
	G	...	..	...	1048	303	71.1
	H	...	..	...	2224	940	57.7
	I	...	..	...	1000	471	52.9
	A	442	28	6.3	1976	1325	33.0
	B	62	7	11.3	2310	1483	35.8
	C	46	5	10.9	3250	1499	53.9
B-6	D	111	33	29.7	2839	583	79.5
2000r	E	28	5	17.9	1900	273	85.6
(1740 rads)	F	...	..	...	417	43	89.7
	G	...	..	...	822	83	89.9
	H	...	..	...	1074	86	92.0
	I	...	..	...	....	....	...
	A	137	13	9.5	586	351	40.1
	B	21	3	14.3	1007	530	47.4
	C	90	9	10.0	1196	357	70.2
B-8	D	14	4	28.6	1090	196	82.0
2000 rads	E	35	3	8.8	1361	100	92.7
	F	...	..	...	352	16	95.5
	G	...	..	...	784	56	92.9
	H	...	..	...	1399	275	80.4
	I	...	..	...	857	409	52.3

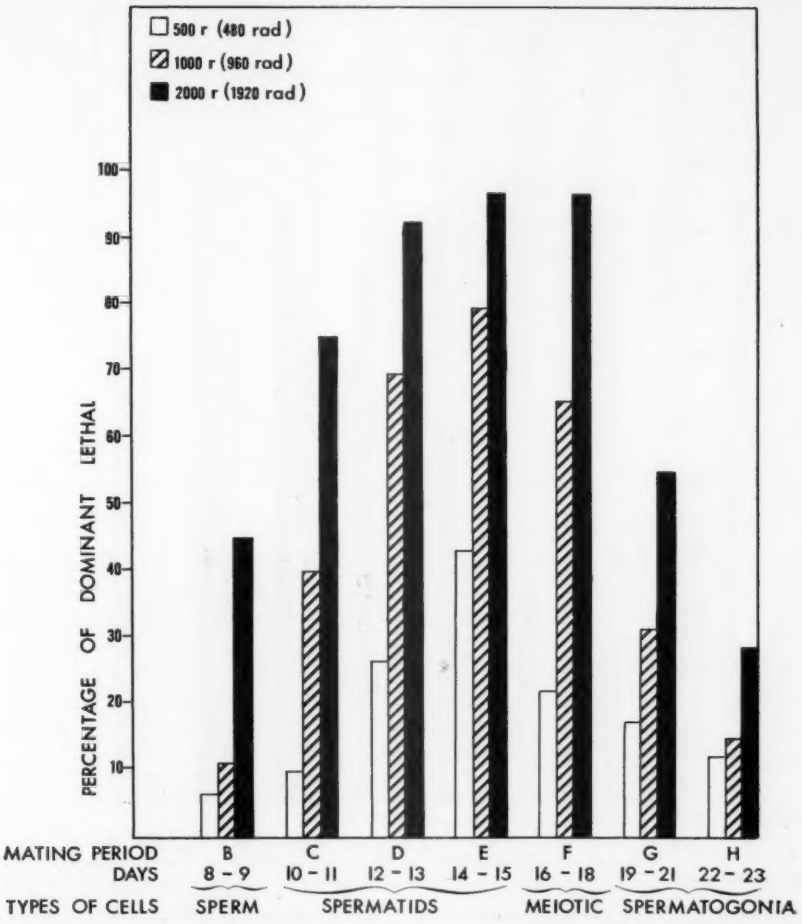

FIGURE 1.—Dominant lethal damage in immature germ cells of *Drosophila virilis* with 200 kv X-ray.

eggs to give an average control value of 94.5 percent for egg development. Each of the control tests, which include remating periods, fell within three percent of the average value of 94.5 percent for all tests. The egg development in the first day's laying period was usually lower than for the second, third or fourth day. The radiation tests also showed a lower value for the first day than for either of the following three days.

For the studies of dominant lethals, normal heterozygous females were used

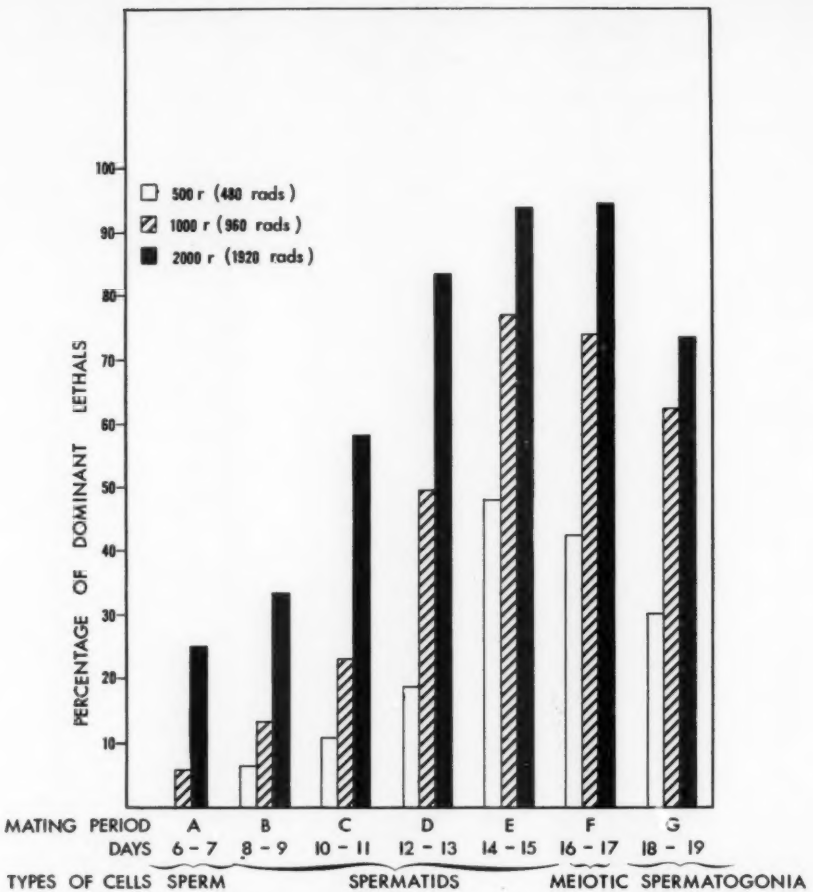

FIGURE 2.—Dominant lethal damage in the immature germ cells of *Drosophila virilis* with 1.17–1.33 Mev gamma rays.

to prevent interference from homozygosity of recessive lethals which might have collected in the standard stocks. Only a very small percent of lethals could result from spontaneous origin of similar lethals in the different strains. However, the control tests showed that not all of the eggs developed into pupae. A sperm fertilization test was used to determine whether some of the eggs which failed to develop had not been fertilized. Eggs were taken, at random, from control tests which were similar to those indicated in Table 4 and also from the 1801.1 standard stock. The eggs were placed on a slide, crushed under a cover-

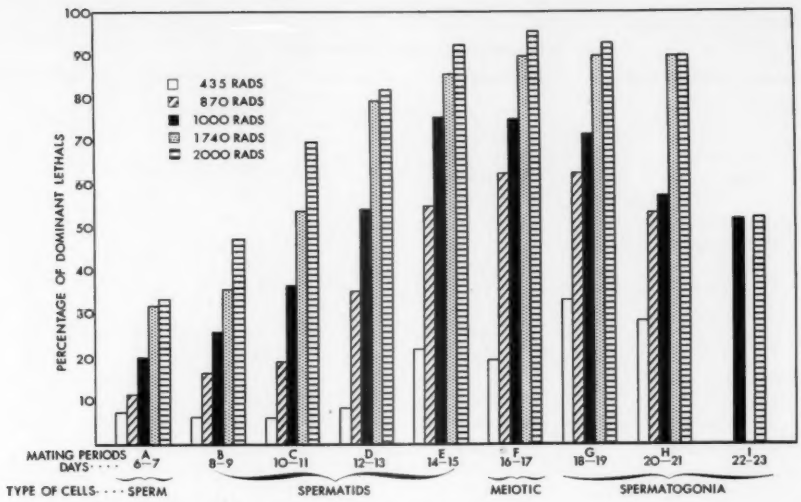

FIGURE 3.—Dominant lethal damage with 22 Mv X-ray.

slip, and checked microscopically for the presence of sperm. In *virilis*, the coiled tails of mature sperm are easily detected in eggs for an hour after eggs have been deposited by the females. When a fertilized egg is crushed, the coiled tails of sperm can be detected usually in the cytoplasm in the micropyle region. The proportion of eggs in which no sperm were observed corresponded to the proportion which failed to develop in the control tests. Apparently the 5.5 percent of eggs which failed to develop resulted from the absence of sperm fertilization of eggs rather than from recessive lethals. The postmeiotic cells in the radiation tests gave similar results.

200 kv X-rays

With 200 kv X-rays, the amount of dominant lethal damage varied in the different types of germ cells of spermatogenesis (Table 1, Figure 1). In postmeiotic cells (periods B-E), the first sample tested, B, contained the lowest percentage of lethal damage. This period represents cells treated as sperm bundles. In cells treated as spermatids, the amount of lethal damage increased and was highest in the most immature spermatids (period E). Although there was an increase in lethal damage in spermatids at all three dose levels, the differences show up better with the 500r (480 rads) test as shown in Figure 1. The postmeiotic spermatids of period E also showed higher percentages of translocations than did the cells of period B. This higher percentage was accompanied by increases in multiple breaks which result in translocations involving more than two chromosomes.

The translocation rate dropped in period F, indicating that germ cells treated

TABLE 4

*Control egg-development tests**

	Laying day				
	1	2	3	4	Total
Mature sperm					
Eggs	2,157	2,696	2,606	2,048	9,507
Percent to develop	84.6	97.3	97.2	98.0	94.5
Young males					
Mating periods A, B, C, D, E					
Eggs	361	759	810	518	2,448
Percent to develop	95.5	97.1	94.4	97.3	96.0
Mating period G					
Eggs	617	466	464	238	1,785
Percent to develop	95.0	98.1	96.6	99.2	96.9
Mating period H					
Eggs	1,077	503	387	905	2,872
Percent to develop	94.4	99.2	93.3	98.3	96.3
Mating period I					
Eggs	324	300	325	579	1,528
Percent to develop	98.8	96.7	97.2	97.1	97.4
Mating period J					
Eggs	213	479	639	295	1,626
Percent to develop	98.6	95.6	96.1	98.6	96.7
Average, all periods					
Eggs	2,592	2,507	2,625	2,535	10,259
Percent to develop	95.6	97.4	95.4	97.9	96.6
Pupae					
Mating periods A, B, C, D, E, F, G					
Eggs	4,944	5,774	4,246	3,625	18,589
Percent to develop	90.7	93.5	95.6	94.2	93.4

* Texmelucan (1801.1) male × female (1999 × 2375.8).

in the meiotic divisions were included in this period. Translocations have not been recovered from cells treated while in meiotic divisions or in premeiotic stages (ALEXANDER 1958a). The percentage of dominant lethals recovered from meiotic cells is lower than that observed for the preceding period of E, with the 500 and the 1000r doses. At these two lower doses, the lethal damage decreased in frequency as treated cells included spermatogonia. With 2000r the lethal damage in meiotic cells was high, the frequency being similar to that obtained for treated spermatids. The frequency of lethals dropped to 55 percent for period G and to 28 percent for period H. A frequency as high as 28 percent of dominant lethals was obtained in matings 22 to 23 days after treatment of the males. In spermatogonial cells increases in lethal damage were produced with increases in dosage. These differences reflect, primarily, differences in induced lethals which survive the meiotic divisions and act as lethals in embryonic development.

The lethal frequencies for the various mating periods are plotted at the three dose levels in Figure 4. The data for periods B, C, D and E indicate an increase

TABLE 5

Dominant lethal damage in mature sperm

Type of radiation	Dose	Experiment	Eggs	Pupae	Percent egg development	Percent dominant lethals
	500r (480 rads)	X-4	2716	2533	93.3	6.7
	1000r (960 rads)	X-5	2730	2430	89.0	11.0
		X-6	2807	1961	69.9	30.1
200 kv	2000r	X-7	15656	9880	63.1	36.9
X-rays	(1920 rads)	X-11	5963	2749	46.1	53.9
		X-19	8547	6002	70.2	29.8
	Totals	(X-6,7,11,19)	32973	20592	62.5	37.5
	3000r (2880 rads)	X-20	7019	3696	52.7	47.3
	500r (480 rads)	G-1	2893	2641	91.3	8.7
1.17–1.33	1000r					
Gamma rays	(960 rads)	G-2	1975	1801	91.2	8.8
	2000r	G-3	5359	4146	77.4	22.6
	(1920 rads)	G-13	9666	6609	68.4	31.6
		G-18	8600	5235	60.9	39.1
	Totals	(G-3,13,18)	23625	15990	67.7	32.3

in sensitivity of postmeiotic cells to radiation treatment, with spermatids (period E) being the most sensitive. All types of immature postmeiotic cells were more sensitive to radiation treatment than mature sperm of seven-day-old males (Table 5). The first period, A, was not included in this experiment although other tests (unpublished) indicate that dominant lethal frequencies for sperm bundles are similar to those for mature sperm. As shown in Figure 4, the frequencies of lethals in meiotic and spermatogonial cells (period F, G, and H) are all lower than the frequency obtained in period E. In period H, the lethal frequencies remained higher than the control levels.

The relationship of the frequency of dominant lethals to increases in the radiation dose (Figure 4) appears to be nonlinear for mating periods B-F which include postmeiotic and meiotic cells. When the data are used for calculations of linear curves with the usual equation of $Y = \alpha + \beta X$, the relationships of the data for some periods to the regression lines are in good agreement although others show imperfect fits. The data for premeiotic cells (period G, H) were more suggestive of linear relationships. The differences in the sensitivity of the

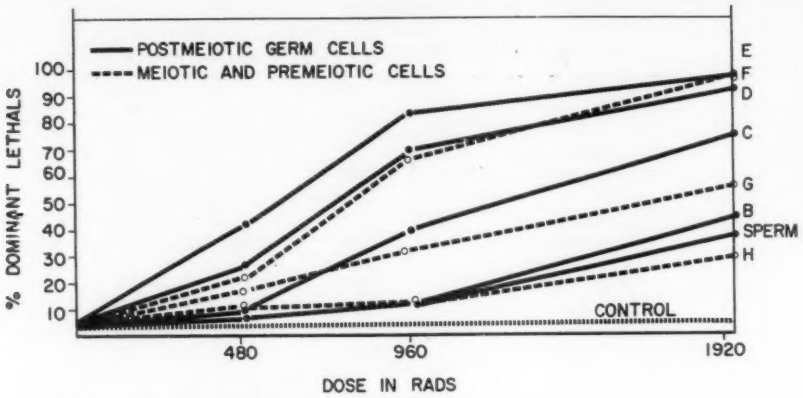

FIGURE 4.—Dominant lethal damage with 200 kv X-rays.

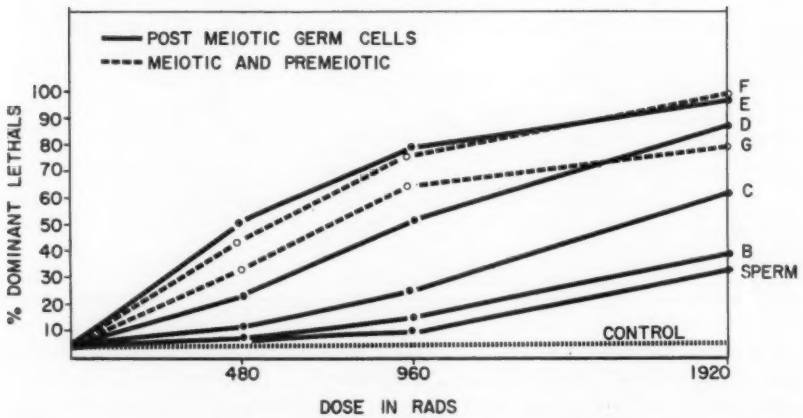

FIGURE 5.—Dominant lethal damage with 1.17–1.33 Mev gamma rays.

various types of cells to the same dose of radiation make it difficult to compare the dose-response curves for different cells using the same three doses of radiation. In some cells, 500r of X-rays produce only eight percent lethal damage whereas in others 50 percent lethals are produced.

1.17–1.33 Mev gamma rays

The frequencies for dominant lethals and translocations produced with gamma-rays are shown in Table 2 and illustrated in Figures 2 and 5. Among the products of postmeiotic cells, lower frequencies of dominant lethals were detected in the progeny of the first mating periods, A and B, than in the later

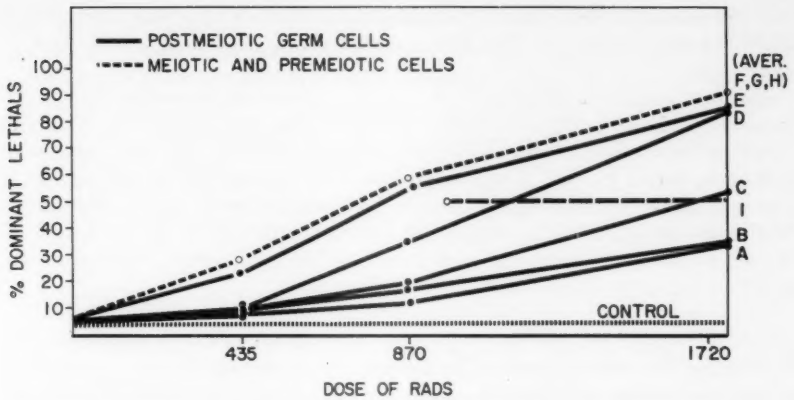

FIGURE 6.—Dominant lethal damage with 22 Mv X-ray from a betatron source.

matings of periods C, D, and E (Figure 2). As with X-rays, young spermatids were more sensitive to radiation than sperm bundles. An increase in translocation frequencies in spermatids was also evident. At 500r, there was an increase from less than one percent translocations to five percent; at 1000r, an increase from two percent to 25 percent; and at 2000r, from three percent to 50 percent. The frequency of dominant lethals remained high in period F and only small numbers of F_1 males were obtained for translocation tests. The lethal frequency remained high through period G where testing was discontinued.

The percentages of dominant lethals are plotted for each dose for cells from all mating periods in Figure 5. The data used to plot the curves for immature cells are included in Table 2. The dominant lethal data for mature sperm of seven-day-old males are included in Table 5. In the first mating period, A of the immature cell tests, dominant lethal frequencies were obtained for two of the three doses and the frequencies were quite similar to those obtained for mature sperm. With respect to dominant lethals, there appears to be no great difference between mature sperm and sperm bundles in response to treatment. Mating periods such as C, D, and E for which the treated cells were predominantly spermatids gave higher lethal values. The meiotic cells (period F) and spermatogonia (period G) have slightly lower lethal frequencies than do the most sensitive spermatid cells of postmeiotic types. The dose-response curves for the various mating periods appear to be of a nonlinear type. The coefficient of correlation to a linear curve varied with most values indicating imperfect relationships.

22 Mv X-rays-betatron

Lethal damage with 22 Mv X-rays was similar to the results obtained with the other two radiations in that among the postmeiotic cells, sperm bundles were less sensitive to radiation than spermatids. The numbers in the translocation

tests were too small for quantitative analyses but the data were extensive enough and consistent enough to show definite increases in translocation damage in spermatids.

The most significant results with betatron treatments were the high lethal frequencies obtained for meiotic and spermatogonial cells (Figure 3). The high values attained in postmeiotic cells were obtained also in meiotic cells (measured in mating period F) and in those cells analyzed through the use of G and H periods. Similar results were obtained in both series of tests with 22 Mv X-rays —with tests B-4, B-5, B-6 and in tests B-7, B-8. The lethal frequencies remained at 50 percent in progeny produced when treated males were remated for an additional two days (period I) in the second series.

In Figure 6, the data for tests B-5, B-6 and B-7 are plotted for each mating period to compare the sensitivity differences in the periods with regard to dominant lethal damage. A gradual, consistent increase in sensitivity was observed among postmeiotic cells as more immature spermatids were represented in the tests. The lethal frequencies for meiotic and spermatogonial cells (F, G, H) were at least equal to or greater than those for spermatids (period E). Since the results for periods F, G and H were so similar, the data for these three periods were averaged for plotting the curve representative of premeiotic periods in Figure 6. The high frequency of dominant lethals in premeiotic cells for 22 Mv X-rays (Figure 6) is distinctly different than those for premeiotic cells with 200 kv X-rays illustrated in Figure 4.

Comparative effectiveness in spermatogenesis

The efficiency of the radiations for producing lethal damage varied with the three radiations. For postmeiotic cells, 200 kv X-rays were more efficient than γ-rays and 22 Mv X-rays. The radiation doses in these experiments allow estimates for 50 percent lethal damage in postmeiotic cells as measured by periods C, D, and E, of meiotic cells from period F, and spermatogonial cells from period G. The calculated doses necessary for 50 percent lethal damage for the three radiations are indicated in Table 6. Relative Biological Efficiencies were calculated from the ratio:

$$\frac{\text{DOSE OF 200 KV X-RAYS FOR 50\% LETHAL DAMAGE}}{\text{DOSE OF GAMMA RAYS OR 22 MV X-RAYS FOR 50\% LETHAL DAMAGE}}$$

Therapy X-rays (200 kv) were more effective than gamma rays and 22 Mv X-rays as shown by the results in the C and D periods. The average values for gamma rays and 22 Mv X-rays are similar although the average for γ-rays is slightly higher. At the stages measured by period E the relative efficiency of γ-rays is similar to that of 200 kv X-rays. In meiotic cells, F, both γ-rays and 22 Mv X-rays were more efficient than 200 kv X-rays. In these germ cells the relative efficiency for producing lethal damage was just reversed to that obtained for postmeiotic cells. Differences in period G were greater and when based on mating periods, gamma rays and 22 Mv X-rays were twice as efficient. The two periods

TABLE 6

Relative efficiency of 200 kv X-rays, gamma rays and 22 Mv X-rays

	Types of radiation	Types of germ cell				
		Postmeiotic			Meiotic	Spermatogonial
		C	D	E	F	G
Doses in rads for 50% lethal damage	200 kv X-rays	1250	750	570	790	1720
	Gamma rays	1720	990	510	590	770
	22 Mv X-rays	1650	1170	810	760	690
Relative biological efficiency	200 kv X-rays / Gamma rays	0.73	0.75	1.11	1.34	2.23
	200 kv X-rays / 22 Mv X-rays	0.76	0.64	0.70	1.05	2.39

F and G represent a total of six days with 200 kv X-rays and only four days for 22 Mv X-rays. This may tend to overestimate the RBE values for G in this test although additional experiments showed that with 22 Mv X-rays the dose for 50 percent dominant lethals remained about the same for periods G, F, and H, or for a period of six days. With 200 kv X-rays the lethal values have dropped to lower values in a comparable period counted in days (F and G). Fifty percent lethal damage was tested in an additional two day period, I, for the betatron radiation with a 1000 rads dose. The estimated dose with the 200 kv X-ray source for 50 percent lethals for the 22–23 day mating period is a minimum estimate of 1720 rads which is twice that required with 22 Mv X-rays (betatron).

DISCUSSION

Variations in several types of biological damage have been reported for different stages of germ cell development of Drosophila. Spermatid types of postmeiotic cells are more sensitive to radiation than are mature sperm. A peak in dominant lethal damage in spermatogenesis was reported for *melanogaster* by LÜNNING (1952). The radiosensitivity peak was shown to include spermatids as well as spermatocytes by AUERBACH (1954). Other types of biological damage such as sex-linked recessives, autosomal recessives, deletions and translocations were also found to be higher in spermatids in her experiments. The high sensitivity of spermatids to radiation effects resulting in translocations and dominant lethals in *virilis* was reported by ALEXANDER, CLAYTON and STONE (1954), and ALEXANDER and STONE (1955). Sensitivity differences in postmeiotic sperm and spermatids have also been observed with several types of radiations. Besides the use of therapy X-rays, biological tests have been obtained in *melanogaster* with X-rays from a betatron linear accelerator source by MOSSIGE (1956) and FRITZ-NIGGLI (1956). With *virilis*, in addition to ionizing radiations such as therapy X-rays, gamma rays and betatron X-rays (ALEXANDER 1957, 1958a), the sensitivity differences for developing germ cells have been reported for fission neu-

trons and accelerator neutrons (ALEXANDER 1956, 1958b,c). Biological damage in spermatids is consistently higher with different biological measures using a series of different types of radiations.

In the present experiments, the relative efficiencies of the three ionizing radations are compared, using the ratio of the doses necessary for lethal damage in 50 percent of the cells. The relative effect for each type of radiation and the effect upon the various types of postmeiotic, meiotic and spermatogonial cells can be compared directly.

The relative biological efficiency (RBE) values calculated for postmeiotic spermatid cells of periods C, D and E are given in Table 6. In postmeiotic cells, the three ionizing radiations, 200 kv X-rays, gamma rays and 22 Mv X-rays, increased the biological damage in spermatids above that for sperm cells. The effectiveness of 200 kv X-rays on spermatids was higher than that obtained with gamma rays or 22 Mv X-rays. The RBE value for the various types of cells using gamma rays, as well as the average value of 0.86, was somewhat higher than the 0.70 value for the betatron.

The RBE values for postmeiotic germ cells of *virilis* with the three radiations correspond generally to the relative physical values of the radiations measured by the Linear Energy Transfer (LET) or by the ion density per micron. Differences in the biological damage with the radiations are not as great as differences in the LET values calculated according to the methods of GRAY (1947) or COMACK and JOHNS (1952). According to GRAY's calculations 200 kv X-rays have a value of 80 ion pairs/μ or a LET value of 2.6 kev/μ; gamma rays from cobalt-60 have a value of 11 ion pairs/μ or a LET value of 0.36 kev/μ and 22 Mv X-rays have a value of 8.5 ion pairs/μ or 0.28 kev/μ LET. According to the biological data reported in Table 6, the average RBE values for cobalt-60 is 0.86 of that for 200 kv X-rays, yet the difference in LET values for X-rays and gamma rays is much greater. Calculations of mean LET values for three radiations by BURCH (1957) include considerations of secondary or δ-track formation. With these calculations the mean LET values per micron of water are 24.4 for therapy X-rays, 19.6 for cobalt-60, and 18.4 for 25 Mv betatron X-rays. The ratio of these values of 1.00: 0.80:0.75 for the quality of the radiations agree more closely with the differences obtained in the biological tests. The RBE values were 1.0:0.86:0.70 for therapy X-ray:γ-rays:22 Mv X-rays in these experiments. The data for mature sperm (unpublished) allow estimates of RBE values but the data are not extensive enough to establish exact values as yet. The RBE values for cobalt and the betatron are no lower than 0.8 and are generally in agreement with the values for spermatids.

The relative efficiencies of the radiations were different when meiotic and spermatogonial cells were sampled by use of periods F and G. Lethal damage in 50 percent of the cells was observed with lower doses of gamma rays and 22 Mv X-rays than with therapy X-rays. With meiotic and premeiotic cells (periods F, G, H), the observed lethal damage and increases in ion density of the radiations showed an inverse relationship. The males were sterile or semisterile when

used in matings 16–19 days (period F, G) after treatment. Semisterility of the males has been shown to be due, in part, to the absence of mature sperm in treated males during certain remating periods (ALEXANDER and STONE 1955). The absence of sperm results from degeneration of spermatogonial cells after radiation treatment. Low frequencies of lethals recovered after therapy X-rays as compared to gamma rays and 22 Mv X-rays imply a differential action for producing cell degeneration in spermatogonia with the various radiations. Those of higher ion densities produce more cell degeneration, and fewer injured cells survive and continue through spermatogenesis for genetic testing. This drop in lethal damage was found to be more noticeable when fission and accelerator neutrons were tested (ALEXANDER 1958b,c). In tests using pupae, all treated postmeiotic and meiotic cells contained lethals, whereas, in premeiotic cells the lethal damage was not significantly higher than in the controls. The differences between a value of 100 percent lethals for one mating period and only one or two percent lethals in the following period make it difficult to explain the differences by chromosome sensitivity alone. The survival of some cells indicates that the presence of resistant cells and differences in sensitivity as measured by mutation rates in sperm and spermatogonial cells occur (ALEXANDER 1954 and unpublished). However, it is difficult to assume that the sensitivity for chromosome breakage can account for a difference this large, and even more difficult to assume that no biological damage has been produced by the doses of neutrons which were used. Since cell degeneration may result after neutron treatment (ALEXANDER 1958b) the selection against certain types of damage by cell degeneration among premeiotic cells most probably occurs. More complete discussions of cell degeneration in Drosophila from radiation treatment appear elsewhere and will not be included here (WELSHONS and RUSSELL 1957; ALEXANDER 1958a).

MOSSIGE (1956) checked recessive lethal damage in the germ cell cycle of *melanogaster* at doses of 1000 and 2500r using 175 kev X-rays and 31 Mev X-rays. The RBE values were calculated by comparing the recessive lethal damage produced by the two radiations. These ratios varied in the cycle with the two radiations although 175 kev X-rays were usually more efficient than 31 Mev X-rays except in mature cells at the lowest dose. At the 2500r dose, there were also variations, and the betatron became more efficient later in the cycle (five days after irradiation). These results could be interpreted in the same way as the *virilis* data since these differences between the therapy X-ray and betatron would be expected at the higher dose in premeiotic cells.

Variations in RBE values for ionizing radiations have been reported in a number of cases. A discussion of cases where conflicting results have been reported with Drosophila appears in EDINGTON and RANDOLPH (1958). Differences in measurement of doses, calculations of dosage, different biological measures of radiation damage and definition of RBE values accounts for some differences.

The RBE values obtained for postmeiotic germ cells of *virilis* agree, in general, with those reported previously for mature sperm of *melanogaster* (for

summary see ZIRKLE 1954). Similar radiations, 200 kev X-rays, 1.25 Mev γ-rays and 23 Mvp X-rays, were used for testing breakage and reunion frequencies in Tradescantia by ARNASON and MORRISON (1954). They found the comparative efficiencies for chromosome breakage with 200 kev X-rays, 1.25 Mev γ-rays and 23 Mvp X-rays to be 1.0:0.82:0.74 in one experiment and 1.0:0.73:0.68 in another. The frequency for chromosome reunion paralleled the breakage frequency fairly closely. CONGER *et al.* (1958) reported RBE values for X- and γ-rays and 1.3-, 2.5- and 14.1 Mev neutrons using Tradescantia. The results for Drosophila (unpublished) agree with their results. The 2.5- and 14.1 Mev neutrons were obtained from the same source for the Drosophila work. The mouse data obtained for the same three ionizing radiations from the same source show similar results. SINCLAIR and BLACKWELL (1958) reported RBE values for the same three sources using LD_{50} doses in mice. They report a ratio of 1.0:0.86:0.82 for 200 kvp X-rays, cobalt-60, γ-rays and 22 Mvp X-rays. The average values for postmeiotic cells in Drosophila are similar to these values. The results with the betatron radiation is somewhat lower than the values obtained with mice. However, this value also appears to be lower than that for mature sperm of Drosophila and the values may be different for spermatids and sperm.

Variations in biological damage involve the environmental concentration of oxygen. The amount of biological damage can be varied by changing the type of cell treated and by changing the type of radiation. Different types of cells respond differently to the same concentration of oxygen. Also neutrons and X-rays produce different amounts of biological damage in similar types of cells with different concentrations of oxygen (ALEXANDER 1958a,b,c).

A consideration of biological damage produced by various radiations is not simply dependent upon the physical characteristics measured by the LET or ion density of the radiations. The physiology of the cells and the interactions of the cell to environmental conditions must be included along with the differences in the physical characteristics of the radiation. When results obtained with neutrons and X-rays are compared, the LET values of different radiations and the biological damage recovered from the radiations agree better with the LET using mature sperm than that for spermatids, meiotic and premeiotic cells. Mature sperm are affected less by environmental changes than are spermatids. When oxygen is eliminated from the environment by treating with nitrogen gas, translocation damage in spermatids is twice that in sperm bundles following treatment either with neutrons or X-rays (ALEXANDER 1958b,c and unpublished). When treatments are carried out in environments of oxygen, the increase in damage to spermatids from neutrons is also doubled, whereas from X-rays, the damage is more than doubled. This is reflected in the RBE values. The RBE ratios become lower for spermatids subjected to the two radiations. For example, when 14 Mev neutrons and 200 kv X-rays are compared with treatments in nitrogen, the neutrons are approximately twice more effective than X-rays in both sperm and spermatids. When comparisons are made of results with treatments in oxygen, the 14 Mev neutrons are again twice more effective in sperm, but both are

equally effective in spermatids. This difference in sperm and spermatids is also obtained when fission neutrons and 200 kv X-rays are compared (ALEXANDER 1958b).

This enhancement by X-rays in spermatids may be explained as due to an increase in the importance of secondary tracks from the primary ion pathways when the opportunity is offered in the biological system. This possibility would require different considerations of secondary tracks with different types of cells. Another consideration is the problem of whether the different types of biological damage are produced by primary or secondary tracks or both. In dividing cells the degeneration of cells from radiation treatment points out another distinct difference in responses which are dependent upon the biological system.

It would be merely an assumption, at the present time, to explain the resulting differences with the three ionizing radiations as due either to quantitative differences with the same mechanism of action for all three radiations, or to different mechanisms for each radiation. The differences obtained with meiotic and spermatogonial cells indicate that the action or actions of the radiations may be different although the observed results of cell lethals in nondividing cells may give a relative effectiveness expected from the LET values of the radiations. The cytological observation of damage using root tips of *Vicia faba* and Ehrlich carcinoma of the white mouse (FRITZ-NIGGLI 1954) showed that with 180 kv X-rays a complete absence of mitotic figures occurred for 36 hours after treatment. With 31 Mev X-rays, mitosis was not suppressed although many of the mitotic figures were abnormal. A different radiobiological action for these two radiations may also be implied from these results.

SUMMARY

Dominant lethal and translocation damage in the mature and immature germ cells of *Drosophila virilis* were observed for ionizing radiations of 200 kv X-rays, γ-rays from the cobalt-60 and 22 Mv X-rays from a betatron source.

Among postmeiotic types of cells, both dominant lethals and translocations were produced with a higher frequency in spermatids than in sperm.

A comparison of the Relative Biological Efficiency of the three radiations in various types of postmeiotic cells showed a higher efficiency of 200 kv X-rays than γ-rays of 22 Mv X-rays. The average RBE values for postmeiotic cells were 1.00:0.86:0.70 for therapy X-rays, γ-rays and betatron X-rays.

The percentages of biological damage produced by the various radiations agree in a quantitative manner with the physical measurements of Linear Energy Transfer or ion density per micron of the radiations. The agreement in the differences in ion density and difference in biological damage depends upon the method for calculating the LET or ion density of the radiation.

The biological results suggest that any one set method will not be sufficient to wholly describe the radiobiological action dependent upon the physical characteristics of the radiation.

Radiation treatment of spermatogonial cells produced different results. Higher

values of lethal damage were observed following 22 Mv X-rays and γ-rays than following therapy X-rays. The 22 Mv X-rays (and γ-rays) were as much as twice as effective in producing lethal damage.

The difference observed for the postmeiotic (nondividing) cells in comparison to dividing cells of meiotic and spermatogonial types is explained as due to a different effectiveness of the radiations for producing cell degeneration in spermatogonial cells. Smaller amounts of lethal damage would be observed for the radiations of higher ion density since a greater proportion of the cells would degenerate and not survive to continue through spermatogenesis for genetic testing.

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A QUANTITATIVE STUDY OF VARIATIONS IN INTENSITY OF GENOTYPES OF THE GUINEA PIG AT BIRTH[1,2]

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THE major uniform color varieties of the guinea pig can be put most conveniently into seven series, each of which ranges from barely distinguishable from white to full intensity.

1. Dark eyed dark sepia	*E aa B P –*
2. Pink eyed pale sepia	*E aa B pp F*
3. Brown eyed dark brown	*E aa bb P –*
4. Pink eyed pale brown	*E aa bb pp F*
5. Pink eyed pale brownish cream	*E aa – pp ff*
6. Stable yellow	*ee – – – F*
7. Fading yellow	*ee – – – ff*

Genes *F*, *f* make no difference in dark sepias (1) or dark browns (3) (with a minor qualification in the latter). No difference between *B* and *bb* has been detected in the pale brownish creams (5). Neither of the pairs *B*, *b* or *P*, *p* make any difference in coat colors of yellows (6,7) at birth but do make a difference in the sootiness that often develops later in yellows. They also make a difference in the color of the skin especially in the ears, feet and nose which are dusky to black with *BP*, brown with *bbP* and very pale with *pp*. Skin color is similar in quality in yellows and nonyellows but less intense in the former. Eye color in yellows depends on *B*,*b*, *P*,*p* in the same ways as in nonyellows, (*CBP* Dark, *CbbP* brown, *C-pp* pink).

Any of the eumelanic colors (1 to 5) may be combined with the corresponding yellow in a tortoise-shell pattern by substitution of e^y for *E*. These same colors may be combined in a banded pattern in each hair (agouti) by substituting *A* for *aa*. This substitution has no effect on yellows at birth, but the presence of *A* is revealed in adult sooty yellows by a clear yellow subterminal band, like that in agoutis. Much of the data of the present paper is derived from tortoise-shells but none from agoutis.

The variations in intensity of these seven series depend primarily on the combinations of the *C* alleles (C, c^k, c^d, c^r, c^a) that are present. *C* (dominant) determines the highest intensity (with qualifications in the case of the dark brown). With $c^a c^a$ there is albinism, white with pink eyes, irrespective of anything else.

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Combinations $c^r c^r$ and $c^r c^a$ reduce yellow to white in the coat but leave the eumelanic colors of coat and eye at intermediate grades. Combinations in which c^d or c^k are the highest alleles bring about different grades of intensity of eumelanin (c^k more intense, c^d somewhat less intense, than c^r) but almost the same grade of yellow at birth. Heterozygotes among c^k, c^d, c^r and c^a are intermediate.

In the colony here studied (largely self contained since 1916) animals have been assigned grades indicating intensity at birth and later, on the basis of standard squares of dorsal skin, chosen so that each grade is barely distinguishable from the preceding. The yellows in the experiments described here ranged from grade 1 barely distinguishable from white to grade 11 for the most intense "red". Modifying factors that increased intensity of *eeCCFF* to grades 12 or 13 at an earlier period, were absent. The sepias and browns are enough alike to be graded on a single scale ranging from 1 to 21 for intense black.

There has been considerable residual variability about the averages established in terms of the preceding factors (WRIGHT 1949). Analysis in the preceding paper (WRIGHT 1959b) indicated that most of this in stable yellows, dark sepias and lower *c* genotypes of dark brown has been due to two pairs of alleles *Si,si* and *Dm,dm*. In the pale sepias and pale browns the effects of these have been overshadowed by unanalyzed factors. The situation in *C* dark browns is peculiar (WRIGHT 1947). The present paper will be concerned with putting the analysis of the effects of gene replacements especially at the *Si* and *Dm* loci on a quantitative basis.

Gene *si* ("silvered") has three somewhat different effects (WRIGHT 1959a). It tends to bring about *silvering* (a sprinkling with white hairs, most pronounced on the posterior belly, least on cheeks and feet). This effect is most conspicuous in blacks (*EBCP*) but is also pronounced in intense browns (*EbbCP*) and pale sepias (*EBCpp*), somewhat less in reds (*eeCFF*), fading yellows (*eeCff*) and dilute sepias ($Ec^d c^d P$). It is usually unrecognizable in the more dilute dark sepias ($Ec^d c^a P$, $Ec^r c^a P$ etc.) and light yellows ($eec^d c^a F$-). Even in blacks the degree of silvering is greatly affected by modifiers and may range from mere traces on the belly to almost white, except for silvered patches on the head and scattered dark hairs on the back. In a typical cross about half the heterozygotes are unsilvered and about half have slight silvering restricted to the belly. In a segregating progeny, heterozygotes overlap both homozygotes. A second effect is *dilution* in unsilvered as well as silvered areas. There is an inverse relation to degree of silvering. Dilution is barely appreciable in blacks, intense browns and pale sepias with *C*. It is more conspicuous in reds and fading yellows and such dilute dark sepias as $EBc^d c^d P$ and dilute yellows as $eec^d c^d FF$. It is most conspicuous in the light sepias ($EBc^d c^a P$) and light yellows ($eec^d c^a FF$). While decidedly less intense than with *SiSi*, these are often as uniform except perhaps for especial lightness of the belly. As a third effect, it is convenient to recognize *blotchiness*. Degree of silvering and less frequently of dilution, tend to be irregular. In high grade silvers, an area that is white except perhaps for scattered dark hairs may be adjacent to an area that is fully colored except perhaps for scattered white hairs.

Blotchiness in intensity may suggest the presence of *sisi* in reds and yellows where no silvering is apparent.

The gene *dm*, "diminished," has little or no effect in the presence of *C* but in lower *c* genotypes brings about a diminution of intensity similar to that due to *si* but lacking the silvering and blotching effects of the latter. In the combination *sisidmdm*, however, the coat becomes pure white, except, in some cases, for spots on the head that are of much reduced intensity and, if *C* is present, strongly silvered. Even eye color is slightly reduced. These "silver-whites" may be looked upon as animals in which the blotching, silvering and dilution effects of *si* have all been carried to an extreme. Silver-whites are anemic and low in viability. The males, as far as known, are always sterile from absence of spermatogenesis and the females are less than half as productive as their normal sisters.

The effects of *si* and *dm* were found to be approximately equal and cumulative in diminishing color in combinations with lower *c* alleles. It will often be convenient to designate the plus alleles of *si* and *dm* merely by + signs, and, for certain purposes to group the combinations according to the number of plus factors: *sisi*+*dm* and +*sidmdm* as class 1, *sisi* ++, +*si* +*dm* and ++*dmdm* as class 2, +*si*++ and +++*dm* as class 3, and ++++ as class 4.

The conclusions on *si* and *dm* indicated above were based largely on the analysis of a large network of matings descended from one (L254) between two extreme dilute yellows that produced silver-whites (both *eeBbc^{d}c^{a}PpFF*, one +*sidmdm*, the other *sisi*+*dm*) and another mating (L263) between an extreme dilute offspring of the preceding and a dilute tortoise-shell of about normal intensity (*eec^{d}c^{a}BbPpFF*+*sidmdm* × *e^{p}ec^{d}c^{a}BBPpFF*+++*dm*). Later outcrosses, especially to an established silvered black-red strain (*e^{p}e^{p}BBCCPPFFsisi*++) also played an important role in the analysis.

All of the matings in this network, including the outcrosses, were assigned formulae in *Si,si* and *Dm,dm* consistent with the intensities of the mated animals and the formulae assigned their parents, with the mean grades and indications of segregation of intensities among the offspring, including especially the occurrence or not of silver-whites, and finally the formulae assigned to all offspring that were mated.

The assignment of genotypes on the basis of routine visual grades was rendered difficult by the smallness of the effects attributed to replacement of a single plus factor by its allele. About one unit was indicated in the dilute yellows, about twice this in the dilute sepias, complicated in the latter by more residual variability. As a difference of one unit is just recognizable by definition, even in direct comparisons, there is room for considerable subjective error.

As litters were graded without reference to the grades in the previous litters, the records for several litters from a mating were deemed reasonably satisfactory in indicating the average level of intensity and in indicating whether segregation, other than that due to *c* genotypes, was or was not occurring within litters.

In the later records from the above network of matings the visual grades were supplemented by fully objective measures of intensity to be discussed presently.

Even where such grades were available, however, there was often doubt about the assignment of formulae. The greatest difficulty was in deciding between *si* and *dm* in parents. A wrong tentative decision would usually be revealed sooner or later by inconsistencies if several offspring were mated, but in the case of a peripheral mating there was often nothing that could be done but compare probabilities. Thus in cases in which it was clear that an animal was transmitting a diminution factor but there was no direct evidence on whether it was *dm* or *si*, the former was preferred on the basis that its frequency was undoubtedly much greater both in the above network of matings and in outcrosses to the main color experiment (in which overt silvering had always been selected against in the rare cases when it appeared). Test matings with the intense silver strain were, however, often made to determine whether key animals were transmitting *si*.

In an earlier series of studies (RUSSELL 1939; HEIDENTHAL 1940; WRIGHT and BRADDOCK 1949) colorimetric determinations had been made of weighed hair samples of animals of many genotypes and these had been correlated with the visual grades of the same animals. These led to formulae by means of which the averages for the much more numerous visual grades for given genotypes could be transformed into quantities of pigment, relative to appropriate standards.

This method of grading was, however, much too slow to be useful in the present connection. The method that was used was that of obtaining galvanometer readings of the intensity of the color in the middle of the back by means of a reflectionmeter (cf. WOLFF 1955; BLAIR 1947). It is possible to transform these to approximate relative quantities of pigment by way of the relation of the visual grades to them and of the colorimetric values to the visual grades.

The purpose of the present paper is to provide estimates based on a large number of readings of this sort for newborn animals of a great variety of genotypes. The results are most complete with respect to the effects of combinations of the *c* genotypes, *Si,si* and *Dm,dm* on dark sepia ($(E,e^p)BP$) and stable yellow ($(e^p,e)F$).

The use of the reflectionmeter in our laboratory (University of Chicago) has been described by WOLFF (1955) who studied the effects of temperature and aging on the coat colors of guinea pigs of many genotypes from these same experiments.

A photovolt photoelectric reflectionmeter, Model 610, was used. This consists of a galvanometer and a search unit containing a light bulb, a photoelectric cell and a choice of three filters, amber, green and blue. It is set to give a reading of zero in the absence of reflected light and 100 for light reflected from a standard enamel plate. A white standard was used for the lighter colors, a gray one for the darker colors. Before taking readings of a litter of guinea pigs with a given filter, checks were made on a darker standard plate and on a black slide box. A freshly washed glass slide was placed on the back of the guinea pig and the search unit was put against this. Light from the bulb reflected from the smoothed hairs to the photoelectric cell resulted in a galvanometer reading. Two readings were taken with each filter and averaged.

The effect of the double passage of the light through the glass slide was found by repeated tests of standard plates, with and without the slide. The relation between the gray and white standards was similarly studied. There were two times during the three years during which records were taken, in which changes in the bulb or wiring required a careful comparison of series of readings made on standard plates before and after. Tables were prepared by means of which a reading made under any set of conditions could be transformed to that for the white standard, no slide on the object and the original bulb and wiring.

On comparison of the galvanometer readings with the visual grades, it was apparent that the scale of the former was a very awkward one. With the darker colors a difference that was very conspicuous to the eye registered only a slight difference on the galvanometer while the opposite was true of the pale colors, from which much light was reflected. The difference from the scale of actual quantities of pigment, as determined colorimetrically, is still greater.

The amounts of variability of single genotypes were found to be more alike at all levels of intensity on the intermediate visual scale than on either the galvanometer readings or the actual amounts of pigment. It accordingly seemed better to transform the galvanometer readings to a scale roughly proportional to the former rather than to the latter. It was found that this could be done by finding the average of the logarithms of the galvanometer readings for the 62 whites that were graded and then subtracting from this the logarithm of the reading for the guinea pig under consideration. This was multiplied by 10 to give numbers of more convenient size. $I_x = 10\ (\overline{log\ R_w} - log\ R_x)$ Tables were prepared for transforming the readings with each filter after reduction to a common standard as described above. As a check, another set of tables was prepared to transform each original reading directly to the scale I_x at a single step. The final figure was thus obtained in every case by two different routes.

The indices from the three filters give a means of describing the qualitative differences among the various coat colors in quantitative terms. The present paper, however, is concerned merely with intensity within each series of colors. The three indices have been added to obtain a single index. $I = I_A + I_G + I_B$ where I_A, I_G and I_B are the indices from the amber, green and blue filters respectively.

The reflectionmeter indices, I, were used in two ways to get estimates of the mean indices for the various genotypes. The first is based on the assignment as accurately as possible of genotypes with respect to *si* and *dm* to each graded individual of a given color series and *c* genotype. The method can be illustrated by considering the case of grades of yellow of genotype $c^d c^d FF$. Grades were taken of tortoise-shells with sufficiently large yellow spots on the back as well as of self yellows.

Indices were obtained for 369 such yellows from 22 types of mating with respect to *si* and *dm* according to the assignment made in analyzing this network of matings. Table 1 gives for each of these types of mating the expected proportions among the offspring of the eight *si dm* genotypes (excluding *sisidmdm* which is

TABLE 1

Types of mating with respect to si *and* dm *that have produced yellows of genotype,* c^dc^dFF. *The expected proportion of each* sidm *genotype, the number of* c^dc^dFF *offspring and their mean indices are shown*

Mating type	4 ++++	3 ++ +dm	3 +si++	2 +si+dm	2 ++dmdm	2 sisi++	1 +sidmdm	1 sisi+dm	Offspring No.	Offspring *I*	*I* (class)
1. ++++ × ++++	1.000	...	...	...	...	...	...	...	21	16.31	16.31
2. ++++ × +++*dm*	.500	.500	...	...	...	...	...	...	4	13.70	
3. ++++ × +*si*++	.500	...	.500	...	...	...	...	...	10	16.80	15.97
4. ++++ × +*si*+*dm*	.250	.250	.250	.250	...	...	...	...	3	14.57	
5. +*si*++ × +*si*++	.250	...	.500	...	...	.250	...	...	14	14.77	14.74
6. ++++ × ++*dmdm*	...	1.000	...	...	...	...	...	...	16	14.93	
7. ++++ × *sisi*++	...	...	1.000	...	...	...	...	...	1	13.80	14.86
8. +++dm × +*si*+*dm*	.125	.250	.125	.250	.125	...	.125	...	17	13.55	13.55
9. ++++ × +*sidmdm*	...	.500	...	.500	...	...	...	...	29	13.09	
10. +++*dm* × ++*dmdm*	...	.500	...	...	.500	...	...	...	54	12.77	12.88
11. +*si*+*dm* × +*si*+*dm*	.067	.133	.133	.267	.067	.067	.133	.133	19	12.42	12.42
12. +++*dm* × +*sidmdm*	...	.250	...	.250	.250	...	.250	...	38	11.98	
13. +*si*++ × +*sidmdm*	...	.250	...	.500	...	...	...	.250	1	9.40	12.05
14. +*si*+*dm* × *sisi*++	...	...	.250	.250	...	.250	...	.250	3	13.70	
15. +*si*+*dm* × +*sidmdm*	...	.143	...	.286	.143	...	.286	.143	5	12.26	12.26
16. ++*dmdm* × ++*dmdm*	...	...	...	....	1.000	...	...	...	68	12.13	
17. ++*dmdm* × *sisi*++	...	...	...	1.000	...	...	...	...	2	10.95	12.09
18. ++*dmdm* × +*sidmdm*	...	...	...	...	.500	...	.500	...	48	11.49	
19. *sisi*++ × *sisi*+*dm*	...	...	...	...	...	.500	...	.500	11	11.98	11.58
20. +*sidmdm* × *sisi*+*dm*	...	...	...	.333	...	...	.333	.333	1	10.50	
21. *sisi*+*dm* × *sisi*+*dm*	...	...	...	...	...	.333	...	.667	3	11.07	10.92
22. ++*dmdm* × *sisidmdm*	...	...	...	...	...	...	1.000	...	1	9.60	9.60
Pure	35.65	69.96	13.75	18.08	119.00	10.00	36.62	8.50			
Mixed	....	7.53	5.40	21.01	13.61	2.02	4.29	3.58			
Grand total	35.65	77.49	19.15	39.09	132.61	12.02	40.91	12.08			
Class total	35.65	96.64			183.72		52.99				

always pure white in the middle of the back). It also shows the number of offspring of genotype $c^d c^d FF$ from which readings of yellow were taken, and the means of the indices. Totals of the expected numbers of each of the *si dm* genotypes are shown below in two categories "pure" and "mixed," and "grand total." Class totals are also shown. The last refers to the number in each class, 1 to 4, according to the number of plus factors. The category "pure" refers to the expectation from matings expected to produce only the genotype in question within its class. The expectations from the matings that could produce two or three *si dm* genotypes in the class in question are called "mixed." In the case of $c^d c^d FF$ yellow, about 53 are expected to have one plus factor (*+sidmdm* or *sisi+dm*), about 184 are expected to have two, about 96 three and about 36 four in the total of 369 graded individuals.

Among these 369 individuals, 77 were mated and accordingly assigned genotypes which took into account their offspring and descendants as well as their own grades and the genotypes assigned their parents. These were entered under the appropriate *si dm* genotype. All of the unmated offspring from matings between homozygotes were next entered under the appropriate genotypes. Among those left, those with the lowest indices from matings expected to produce class 1 were assigned to *+sidmdm*, *sisi+dm* or mixed according to the possibilities from the mating in question until the quota of 53 for class 1 was filled. The pure and mixed subdivisions of class 2 were then filled with those of the lowest indices not already assigned, from matings expected to produce this class, until its quota of 153 was complete. The classes with three plus factors were filled similarly, up to its quota of 96, leaving 36 in class 4.

In spite of the somewhat arbitrary assignment, in part by rank, there was considerable overlap because of mated individuals whose assigned genotypes were somewhat out of line, and because of individuals that were somewhat out of line with respect to possibilities provided by the genotypes assigned their parents. Nevertheless one might expect this method to be subject to a systematic error in the direction of spreading the averages. What it gives are upper limits to the estimates of the differences among the means, apart from errors in the assignment of genotypes to the matings.

Table 2 shows for yellows of each *c* genotype, the assigned number (n_o), the expected number (n_e) and the mean index pertaining to each class and subclass with respect to *si* and *dm*. The sampling errors for intense yellows (*C*) may be taken as $\sqrt{1.73/n_o}$ and for all lower *c* combinations as $\sqrt{.79/n_o}$ on the basis of pooled variances presented in Table 4.

It may easily be seen that there are highly significant differences between classes under any given *c* genotype. There are, however, few differences within classes on which any confidence can be based. The most striking is between the silvered reds of genotype *Csisi* ++ ($20.8 \pm .38$) and the unsilvered ones of genotypes *C++dmdm* ($23.9 \pm .58$) and *C+si+dm* ($22.5 \pm .58$). These unsilvered reds of class 2 are as intense as reds of classes 3 or 4. Genotype *C++dmdm*, based unfortunately on offspring of only one mating (LC29), is actually more

TABLE 2

Classes and subclasses with respect to si *and* dm *for yellows* (FF) *for the various* c *genotypes and grouping of these. The number* (n_o) *assigned each subclass and the number* (n_e) *expected on the basis of such tables as Table 1 are shown and also the mean indices of those assigned in each case*

	Yellow	C n_o (n_e)	Mean	c^bc^b n_o (n_e)	Mean	c^bc^d n_o (n_e)	Mean	c^bc^r n_o (n_e)	Mean	c^bc^a n_o (n_e)	Mean	$c^bc^r + c^bc^a$ n_o (n_e)	Mean
4	++++	18(25.9)	22.2	15(14.7)	14.6	15(14.6)	16.6	10(7.3)	10.7	15(17.2)	10.0	25(24.5)	10.3
3	+++*dm*	.....	...	18(18.5)	13.7	8(13.5)	14.4	13(14.5)	9.0	29(30.7)	9.0	42(45.2)	9.0
	+*si*++	.....	...	.....	...	..(3.6)	...	.....	...	..(4.5)	...	..(4.9)	...
	mixed	47(39.0)	23.0	.....	...	9 ...	14.9	.....	...	8 ...	9.0	8 ...	9.0
2	++*dmdm*	9(13.0)	23.9	8(7.8)	12.3	13(10.9)	12.8	6(7.2)	7.4	26(26.9)	7.2	32(34.1)	7.3
	+*si*+*dm*	9(17.9)	22.5	.....	...	1(4.5)	11.2	.....	...	2(9.1)	7.8	2(9.1)	7.8
	sisi++	20(20.4)	20.8	.....	...	..(0.0)	...	.....	...	..(0.1)	...	..(0.1)	...
	mixed	13	21.9	.....	...	1 ...	12.7	.....	...	9 ...	7.1	9 ...	7.1
1	+*sidmdm*	5(4.7)	19.8	.....	...	1(0.9)	9.4	.....	...	5(4.7)	5.3	5(4.7)	5.3
	sisi+*dm*	16(16.1)	17.8	.....	...	..(0.0)	...	.....	...	..(0.4)	...	..(0.4)	...
	mixed	.....	...	.....	...	.....	...	.....	...	.....	...	.....	...
	Total	137 ...	...	41 ...	...	48 ...	...	29 ...	...	94 ...	...	123 ...	...

		c^dc^d n_o (n_e)	Mean	c^dc^r n_o (n_e)	Mean	c^dc^a n_o (n_e)	Mean	c^dc^r or c^dc^a n_o (n_e)	Mean	$c^dc^r + c^dc^a$ n_o (n_e)	Mean		
4	++++	36(35.7)	16.5	2(2.6)	10.8	24(23.3)	10.7	.....	...	26(25.3)	10.7	.....	...
3	+++*dm*	70(77.5)	14.1	8(11.0)	8.4	18(23.2)	9.1	1(1.0)	8.9	27(35.2)	8.9	.....	...
	+*si*++	11(19.2)	14.9	..(1.4)	...	15(23.2)	9.1	.....	...	15(24.6)	9.1	.....	...
	mixed	15 ...	14.4	8 ...	9.1	10 ...	9.0	.....	...	18 ...	9.0	.....	...
2	++*dmdm*	131(132.6)	12.1	23(22.9)	6.8	53(53.6)	6.1	5(7.2)	6.9	81(83.7)	6.4	.....	...
	+*si*+*dm*	17(39.1)	12.0	1(2.3)	6.1	14(22.5)	7.0	..(1.5)	...	15(27.3)	6.9	.....	...
	sisi++	14(12.0)	13.1	..(0.0)	...	6(10.5)	6.0	..(0.0)	...	6(10.9)	6.0	.....	...
	mixed	22 ...	12.4	2 ...	6.7	15 ...	6.0	3 ...	6.4	20 ...	6.1	.....	...
1	+*sidmdm*	37(41.9)	10.4	2(4.5)	4.2	34(42.6)	3.3	4(3.3)	2.8	40(50.4)	3.3	.....	...
	sisi+*dm*	10(12.1)	10.1	..(0.3)	...	15(13.7)	3.4	..(0.0)	...	15(14.0)	3.4	.....	...
	mixed	6 ...	10.7	.. ...	...	9 ...	3.5	.....	...	9 ...	3.5	.....	...
	Total	369 ...	...	46 ...	...	213 ...	...	13 ...	...	272 ...	...	.....	...

TABLE 3

Classes and subclasses with respect to si *and* dm *for dark sepias* (BP) *for the various* c *genotypes and groupings of these. The number assigned, the number expected from the mating types and the mean indices of those assigned are shown*

	Sepia	C n_a (n_e)	Mean	c^bc^b n_a (n_e)	Mean	c^bc^d n_a (n_e)	Mean	c^bc^r n_a (n_e)	Mean	c^bc^b, c^bc^d or c^bc^r n_a (n_e)	Mean	$c^bc^b + c^bc^d + c^bc^r$ n_a (n_e)	Mean
4,3	.....	66(66.2)	41.3	33 ...	42.5	4(2.5)	41.4	25 ...	41.1	9 ...	41.3	71(71.8)	41.2
2	++*dmdm*	.....	...	5 ...	39.3	1(0.6)	38.1	3 ...	40.7	8 ...	38.5	17(15.3)	39.1
	+*si*+*dm*	.....	...	.....	...	..(0.2)	...	.....	...	.....	...	..(1.2)	...
	+*sidmdm*	2 ...	38.9	.....	...	1(0.6)	37.2	.....	...	.....	...	1(0.6)	37.2
1	*sisi*+*dm*	10 ...	40.3	.....	...	.....	...	.....	...	.....	...	.....	...
	mixed	2 ...	39.5	.....	...	.....	...	.....	...	.....	...	.....	...
	Total	80 ...	...	38 ...	...	6 ...	...	28 ...	...	17 ...	...	89 ...	...

		c^bc^a n_a (n_e)	Mean	c^dc^d n_a (n_e)	Mean	c^dc^r n_a (n_e)	Mean	c^dc^a n_a (n_e)	Mean	c^rc^r n_a (n_e)	Mean	c^rc^a n_a (n_e)	Mean
4	++ ++	14(14.0)	40.1	6(6.3)	38.0	7(6.7)	39.9	23(22.9)	34.1	11(26.0)	40.2	21(21.3)	34.6
	+++*dm*	29(30.1)	38.5	6(7.8)	36.0	4(6.5)	39.0	20(27.8)	31.1	6(23.0)	39.9	33 ...	33.6
3	+*si*++	..(1.7)	...	5(6.3)	37.1	4(3.5)	37.8	1(6.5)	30.2	.....	...	3 ...	32.5
	mixed	3 ...	38.1	3 ...	35.9	1 ...	38.5	13 ...	31.1	32 ...	40.7	11 ...	33.1
	++*dmdm*	8(15.5)	35.6	31(32.1)	35.0	9(8.8)	37.7	35(41.9)	27.7	2(2.5)	39.2	25 ...	29.1
2	+*si*+*dm*	3(5.6)	36.6	6(9.8)	34.9	..(4.8)	...	19(25.0)	28.2	3(2.2)	39.0	11 ...	29.6
	sisi++	..(0.0)	...	7(3.6)	35.1	..(0.0)	...	5(3.7)	26.1	.....	...	.....	...
	mixed	10 ...	36.2	2 ...	34.6	5 ...	37.1	12 ...	27.1	.....	...	32 ...	29.9
	+*sidmdm*	5(5.9)	31.7	9(10.6)	31.5	8(6.3)	35.5	35(37.4)	23.1	..(1.7)	...	28 ...	25.1
1	*sisi*+*dm*	..(0.6)	...	4(4.4)	32.3	..(1.3)	...	4(6.8)	21.8	..(1.6)	...	4 ...	25.1
	mixed	1 ...	34.3	.....	...	.....	...	5 ...	22.1	3 ...	35.9	5 ...	24.4
	Total	73 ...	...	79 ...	...	38 ...	...	172 ...	...	57 ...	...	173 ...	...

TABLE 4

Intraclass variances (σ^2) and degrees of freedom for indicated genotypes

Yellow (*ee*) Genotype	d.f.	σ^2	Dark sepia (*EBP*) Genotype	d.f.	σ^2	Pale sepia (*EBppFF*) Genotype	d.f.	σ^2
C FF	134	1.73	*C*	78	1.70	*C*	62	13.79
c^kc^kFF	38	.65	c^kc^{kdr}	88	1.07	c^kc^{kd}	28	5.31
c^kc^dFF	44	.79	*C*, c^kc^{kdr}	166	1.36	c^kc^{ra}	22	4.76
$c^kc^{ra}FF$	119	.36	c^kc^a	69	1.38	c^dc^d	37	1.80
c^dc^dFF	365	.82	c^dc^d	75	1.95	c^dc^a	66	3.25
$c^dc^{ra}FF$	268	.94	c^dc^r	34	1.43	c^rc^r	40	4.33
FF (excl *C*)	834	.79	c^dc^a	168	2.11	c^rc^a	16	2.85
C Ff	38	2.76	c^rc^r	54	2.00	excl *C*	209	3.60
$c^{kd}c^{kd}Ff$	5	1.66	c^rc^a	169	2.08			
C ff	54	2.94	excl *C*,c^kc^{kdr}	569	1.94			
$c^{kd}c^{kd}ff$	12	4.55						

Pale brownish cream (*Eppff*) Genotype	d.f.	σ^2	Dark brown (*EbbP*) Genotype	d.f.	σ^2	Pale brown (*EbbppFF*) Genotype	d.f.	σ^2
ECppff	121	3.49	*C* (non *dn*)	197	3.57	*C*	37	4.80
			c^k-	10	1.07	c^dc^{dr}	31	3.32
			c^dc^d	25	3.95	c^dc^a	37	3.60
			c^dc^a,c^rc^a	47	1.94	c^rc^{ra}	25	2.36
			excl *C*	82	2.45	excl *C*	93	3.13

intense than yellows of any other category. It seems that $++dmdm$ and probably $+si+dm$ cause no diminution of intense yellow whatever while $sisi++$ causes appreciable diminution even in the unsilvered areas in which the grades were taken. There is a parallel difference between $sisi+dm$ ($17.8 \pm .43$) and $+sidmdm$ ($19.8 \pm .77$) but it is of doubtful significance. Among the c^dc^d yellows there is an apparently significant difference between the silvers ($13.1 \pm .21$) and the others of class 2 ($12.1 \pm .07$) but it is in the opposite direction from that in the *C* yellows. There is a suggestion here that *dm* interacts specifically with the lower *c* alleles in diminishing their intensity while *si* acts more uniformly with all *c* alleles. The scanty data in other cases do not, however, add support to this idea.

Table 3 presents similar data for the dark sepias. The standard errors of blacks (C,c^kc^{kdr}) may be taken as $\sqrt{1.36/n_o}$ and for the other dark sepias as $\sqrt{1.94/n_o}$ from the pooled variances in Table 4. There are no differences within classes of the same *c* genotype that are significant. This holds also for the scanty data from the remaining color series.

From this point all data will be grouped by classes except in the one case of intense yellows of class 2 ($sisi++$ vs. $+si+dm$ and $++dmdm$ collectively).

The second method of analyzing the data has been to make estimates of the means of the classes of *sidm* genotypes by the method of least squares. Tables like 5 (for c^dc^dFF yellows) were prepared for each *c* genotype. All types of mating that give the same proportion of offspring by classes are here combined. Thus in Table 5, the 22 types of matings of Table 1 are reduced to 13. The table is in the

TABLE 5

Matings that produce yellows of genotype c^dc^dFF, *classified according to the expected ratios with respect to modifier classes, the observed mean indices from each, the expected mean from estimation of the effects of the modifiers and the differences between observed and expected means. The weights* (w) *are as described in the text*

Yellow *n*	c^dc^dFF *w*	Class (no. of plus factors) (4)	(3)	(2)	(1)		m_o	m_e	m_o-m_e
21	21.0	$\ldots m_4$	$\ldots$	$\ldots$	$\ldots$	=	16.31	16.41	− .10
14	6.7	$.500\,m_4 +$	$.500\,m_3$	$\ldots$	$\ldots$	=	15.97	15.47	+ .50
17	5.3	$.250\,m_4 +$	$.500\,m_3 +$	$.250\,m_2$	$\ldots$	=	14.74	14.36	+ .38
17	4.1	$.125\,m_4 +$	$.375\,m_3 +$	$.375\,m_2 +$	$.125\,m_1$	=	13.55	13.35	+ .20
19	4.5	$.067\,m_4 +$	$.266\,m_3 +$	$.400\,m_2 +$	$.267\,m_1$	=	12.42	12.65	− .23
17	17.0	$\ldots$	$\ldots m_3$	$\ldots$	$\ldots$	=	14.86	14.52	+ .34
83	39.5	$\ldots$	$.500\,m_3 +$	$.500\,m_2$	$\ldots$	=	12.88	13.26	− .38
42	14.0	$\ldots$	$.250\,m_3 +$	$.500\,m_2 +$	$.250\,m_1$	=	12.05	12.34	− .29
5	1.7	$\ldots$	$.143\,m_3 +$	$.428\,m_2 +$	$.429\,m_1$	=	12.26	11.86	+ .40
70	70.0	$\ldots$	$\ldots$	$\ldots m_2$	$\ldots$	=	12.09	12.00	+ .09
59	31.1	$\ldots$	$\ldots$	$.500\,m_2 +$	$.500\,m_1$	=	11.58	11.41	+ .17
4	2.2	$\ldots$	$\ldots$	$.333\,m_2 +$	$.667\,m_1$	=	10.92	11.22	− .30
1	1.0	$\ldots$	$\ldots$	$\ldots$	$\ldots m_1$	=	9.60	10.83	−1.23
Σnp 269	...	35.7	96.6	183.7	53.0				
Σwp ..	218.1	26.5	49.2	118.4	24.0				

form of a series of observation equations in each of which the sum of the unknown means for the various classes (m_1, m_2, m_3, m_4), each multiplied by the expected proportion of offspring in its class (excluding *sisidmdm*) is equated to the observed mean for the type of mating in question. The weights to be assigned these equations should be proportional to the reciprocals of the squared standard errors of their means. As some of the theoretical variances are based on very small numbers, it seemed best to use estimates of the theoretical variances, taking account of both the variances within and among classes. Estimates of the latter were obtained by using the estimates of the means obtained by the first method. As variances of these were expected to be somewhat too large and those within the classes somewhat too small, because of the mode of assignment of individual genotypes, the estimates of the variance within classes were arbitrarily increased 25 percent. The results seem to indicate that this was too large a correction, but the effect of slight difference in the relative weights of the equations can have no appreciable effect on the result, at the level of precision that the data permit. The most important factor in a weight remains the number of offspring from the type of mating in question. The intraclass variances used were 2.2 for intense yellows (C), 1.0 for the dilute yellows, 1.7 for the blacks (C, c^kc^{kdr}) and 2.5 for the other dark sepias. The numbers of offspring and the weights (w) in the case of the c^dc^dFF yellows are given in the first two columns of Table 5. It may be seen that the weights vary from about one fourth of the number in the

cases with the widest segregation, to equality with the number in the cases with no segregation at all.

This method, in contrast with the first, has no systematic error. On the other hand, it loses all of the information given by the actual segregation among the offspring of the types of mating. Both methods are of course affected by mistakes in the assignment of genotypes. Since this assignment is partly based on the mean intensities of the offspring there is to some extent circular reasoning in deducing the means of genotypes from them. This is greatly mitigated, however, by the fact that the progeny means of the reflectionmeter indices with respect to a particular color (here yellow) and a particular *c* genotype (here $c^d c^d FF$) is, as previously noted, only a small part of the information on which genotypes are assigned to the parents.

The commonest type of error in assigning *si dm* genotypes was probably in replacing one of the same class by another (e.g., $+si++$ by $+++dm$). Such errors would blur the differences in the effects of *si* and *dm* indicated by the first method, but would have little effect on the class means estimated by the second method, assuming that these differences are small.

The least square method tends to break down where the total expected number of any class is very small, since in this case this class may not actually have been present at all, or if present from one or two types of mating, its effect on the means may be overbalanced by accidental difference in the ratio of the other genotypes. Thus in the $c^k c^d$ yellows the expected number in class 1 is only 0.9. Just one $+sidmdm$ was probably present, as indicated by a single outstandingly light segregant (index 9.4) but the mating type in which this occurred had such an unexpectedly high average that the solution for m_1 comes out higher than for m_2 and m_3. It seems best to drop m_1 from the parameters to be estimated in this case and revise the expected proportions from the mating type expected to produce 12.5 percent $+sidmdm$ in accordance and revise the mean by omitting the one probable representative of this genotype. Similarly the expected number of $+sidmdm$ for $c^k c^{kdr}$ blacks is 0.6. There was again one offspring from the one mating type expected to produce this that was much lighter than all others (37.2). Again class 1 and its probable representative were omitted from the least square estimate.

In a number of cases the least square estimates for m_3 and even m_2 came out about the same or slightly larger than m_4. These were cases in which there was probably little or no real difference and also ones in which the evidence for assigning genotypes was most deficient. It seemed best, accordingly, to group classes 2, 3 and 4 in the case of *CFF* yellows (except for $sisi++$). Similarly classes 3 and 4 are grouped in $c^k c^k$ yellows, classes 2, 3 and 4 in the case of *C*-blacks and classes 3 and 4 in the cases of sepias of genotypes $c^k c^{kdr}$, $c^k c^a$, $c^d c^r$, $c^r c^r$ and $c^r c^a$. These are also grouped in the final estimate by the first method since the small difference found originally could well have been imposed by the method.

Standard errors for the least square estimate were obtained by the formula

$SE = \sqrt{\Sigma w\,(m_o - m_c)^2 / K\,\Sigma wp}$ where K is the number of degrees of freedom among mating types, p is the proportion of the given class expected from each mating type, w is the weight and m_o and m_c are the observed and estimated means for the mating types. The values of Σwp are shown in the bottom row of Table 5 (case of $c^d c^d FF$ yellows).

Tables 6 and 7 give comparisons of the mean indices for the various genotypes of yellows and dark sepias respectively derived by the two methods. The results

TABLE 6

Comparison of estimates of mean indices for the various genotypes of yellows based (1) on individual assignment of genotypes and (2) on least squares from the means of mating types. The differences and a weighted average are shown. The corresponding visual grades are estimated from these averages and compared with 1949 visual grades. The amounts of pigment are estimated from these.

Yellow class	No.	Reflectionmeter indices (1) Individuals	Reflectionmeter indices (2) Means	Diff.	Av. *I*	Visual grades From *I*	Visual grades 1949	Pigment From *I*	Pigment 1949
C FF 4,3	65	22.8 ± .16	22.6 ± .23	+ .2	22.7	10.5	10.9	100.0	100.0
2(excl *sisi*)	31	22.7 ± .24							
2(*sisi*++)	20	20.8 ± .29	20.3 ± .54	+ .5	20.7	9.7	...	80.7	...
1	21	18.3 ± .29	19.3 ± .63	−1.0	18.5	8.7	...	63.3	...
$c^k c^k$ 4,3	33	14.1 ± .16	14.4 ± .23	− .3	14.2	6.9	7.0	37.9	36.1
2	8	12.2 ± .31	10.6 ± .53	+1.6	11.8	5.8	...	27.6	...
$c^k c^d$ 4	15	16.5 ± .23	16.3 ± .41	+ .2	16.5	7.9	...	50.2	...
3	17	14.7 ± .22	14.0 ± .52	+ .7	14.6	7.0	7.2	39.8	38.6
2	15	12.7 ± .23	13.5 ± .47	− .8	12.9	6.3	...	32.0	...
1	1	9.4 ± .90	.....	....	9.4	4.8	...	19.3	...
$c^k c^{ra}$ 4	25	10.3 ± .18	9.7 ± .20	+ .6	10.0	5.1	...	21.2	...
3	50	9.0 ± .13	9.3 ± .14	− .3	9.1	4.7	4.7	18.4	17.5
2	43	7.3 ± .14	7.3 ± .15	0	7.3	3.9	...	13.4	...
1	5	5.3 ± .40	5.4 ± .36	− .1	5.3	3.0	...	8.8	...
$c^d c^d$ 4	36	16.4 ± .14	16.4 ± .25	0	16.4	7.8	...	49.6	...
3	96	14.3 ± .09	14.5 ± .19	− .2	14.3	6.9	6.9	38.4	35.7
2	184	12.2 ± .07	12.0 ± .12	+ .2	12.2	6.0	...	29.1	...
1	53	10.4 ± .12	10.8 ± .27	− .4	10.5	5.3	...	22.9	...
$c^d c^{ra}$ 4	26	10.7 ± .18	11.1 ± .31	− .4	10.8	5.4	...	23.9	...
3	60	9.0 ± .12	9.0 ± .25	0	9.0	4.6	4.2	18.1	13.8
2	122	6.4 ± .08	6.7 ± .15	− .3	6.5	3.6	...	11.5	...
1	64	3.4 ± .11	2.5 ± .26	+ .9	3.2	2.1	...	4.8	...
C Ff	39	20.6 ± .27	.....	....	...	9.6	10.0	79.8	85.3
$c^k c^k Ff$	3	11.2 ± .30	.....	....	...	5.6	6.5	25.3	31.0
$c^d c^d Ff$	3	10.3 ± .30	.....	....	...	5.2	6.7	22.2	32.9
C ff	55	13.2 ± .23	.....	....	...	6.4	6.8	33.3	34.5
$c^k c^k ff$	3	2.4 ± 1.23	.....	....	...	1.8	1.9	3.5	4.5
$c^d c^d ff$	10	2.6 ± .67	.....	....	...	1.9	2.3	3.8	5.1
ECffpp	122	5.0 ± .16	.....	....	...	2.9	2.3	8.2	6.2

TABLE 7

Mean indices for various genotypes of dark sepia and derived estimates as in Table 6

Dark sepia class		(*BP*) No.	Reflectionmeter indices (1) Individuals	Reflectionmeter indices (2) Means	Diff.	Av. *I*	Visual grades From *I*	Visual grades 1949	Pigment From *I*	Pigment 1949
C	4,3,2	66	41.3 ± .14	41.3 ± .25	0	41.3	20.9	20.9	100.0	100.0
	1	14	40.0 ± .31	39.7 ± .58	+ .3	39.9	19.9	...	87.1	...
c^kc^{kdr}	4,3	71	41.2 ± .14	41.0 ± .14	+ .2	41.1	20.8	20.2	98.1	89.9
	2	17	39.1 ± .28	39.8 ± .30	− .7	39.4	19.6	...	82.9	...
	1	1	37.2 ± 1.17	.....	....	37.2	18.0	...	66.4	...
c^kc^a	4,3	46	39.0 ± .20	38.9 ± .44	+ .1	39.0	19.3	18.9	79.6	74.7
	2	21	36.0 ± .31	36.5 ± .72	− .5	36.1	17.2	...	59.4	...
	1	6	32.1 ± .57	31.3 ± 1.52	+ .8	32.0	14.7	...	40.8	...
c^dc^d	4	6	38.0 ± .57	37.8 ± .92	+ .2	38.0	18.6	...	72.0	...
	3	14	36.4 ± .37	36.9 ± .69	− .5	36.5	17.5	18.1	61.9	66.6
	2	46	35.0 ± .21	35.2 ± .35	− .2	35.0	16.5	...	53.0	...
	1	13	31.7 ± .39	31.3 ± .94	+ .4	31.7	14.6	...	39.9	...
c^dc^r	4,3	16	39.1 ± .35	38.7 ± .33	+ .4	38.9	19.2	18.7	78.8	72.1
	2	14	37.5 ± .37	37.4 ± .37	+ .1	37.4	18.2	...	67.8	...
	1	8	35.5 ± .50	36.3 ± .53	− .8	35.9	17.1	...	58.2	...
c^dc^a	4	23	34.1 ± .29	33.6 ± .38	+ .5	33.9	15.7	...	47.2	...
	3	34	31.0 ± .24	31.6 ± .41	− .6	31.2	14.3	14.5	38.6	32.7
	2	71	27.6 ± .17	27.2 ± .26	+ .4	27.5	12.6	...	29.5	...
	1	44	22.8 ± .21	23.2 ± .40	− .4	22.9	10.5	...	20.7	...
c^rc^r	4,3	49	40.5 ± .20	40.5 ± .07	0	40.5	20.3	19.5	92.4	81.2
	2	5	39.1 ± .63	40.4 ± .25	−1.3	40.3	20.2	...	90.6	...
	1	3	35.9 ± .81	35.5 ± .34	+ .4	35.6	16.9	...	56.4	...
c^rc^a	4	21	34.6 ± .31	33.4 ± .20	+ .4	33.6	15.5	15.0	45.8	42.4
	3	47	33.4 ± .20							
	2	68	29.6 ± .17	30.5 ± .25	− .9	29.9	13.7	...	35.1	...
	1	37	25.0 ± .23	24.2 ± .35	+ .8	24.8	11.4	...	24.0	...

of the least square estimate from mating type means may be seen to be rather close to those obtained from assignment of genotypes to individuals, in both tables.

In most cases, the sampling standard errors are much larger for the least square estimates as expected from the loss of information. In some cases, however, they came out about the same, presumably because of accidentally small values of $\Sigma w(m_o - m_c)^2$ in the least square standard errors.

The range from class 1 to class 4 is larger in the estimates from individual assignments in, for example, the case of c^dc^dFF yellows (6.07 *vs.* 5.59), as expected, because of the systematic error in using rank as a criterion. There is, however, no consistent difference in spread. It appears that the amount of systematic error in the first method was not important.

It is desirable to obtain a single estimate for each genotype. The estimates from the individual assignments use all the data but are subject to systematic error. On the other hand those obtained by the method of least squares are not subject to systematic error but do not use all the information. The differences are, however, so slight (after making the groupings noted above) that it makes little difference in most cases which is used. There is so little indication of systematic error in the first estimates that they might well be used. The averages weighted by the reciprocals of the squared standard errors of sampling make considerable use of the least square estimates where these are most reliable and make little use of them otherwise. These are shown in Tables 6 to 10 (Av. *I*). They do not differ in any case from those from the first method by amounts that can be considered important but may be supposed in general to give a slight correction of the systematic error of the method. The sampling standard error cannot of course be less than by the first method, based on individuals.

Visual grades in relation to reflectionmeter indices

It is desirable to compare these estimates with those obtained previously (WRIGHT 1949) from visual grades and from colorimetry in which the *si dm*

TABLE 8

Mean indices for various genotypes of dark brown and derived estimates as in Table 6

Dark brown class	(*bbP*) No.	Reflectionmeter indices (1) Individuals	(2) Means	Diff.	Av. *I*	Visual grades From *I*	Visual grades 1949	Pigment From *I*	Pigment 1949	Percent *B* (*I* as if *B*)
CP F	198	34.8 ± .13	.....	....	...	15.4	15.3	100.0	100.0	49.5
$c^k c^{kdr}$ 4,3(2)	8	34.6 ± .55	.....	....	...	15.3	15.1	98.6	97.0	48.8
$c^k c^a$ 4	6	29.1 ± .63	.....	....	...	12.9	14.1	67.9	83.0	33.2
$c^d c^d$ 4	4	33.2 ± .81	33.7 ± .97	− .5	33.4	14.8	...	91.1	...	45.0
3	4	31.5 ± .81	30.6 ± 1.07	+ .9	31.2	13.8	14.2	78.5	84.6	38.6
2	17	30.1 ± .39	30.2 ± .41	− .1	30.1	13.3	...	72.8	...	35.6
1	4	26.5 ± .81	25.2 ± 1.24	+1.3	26.1	11.5	...	54.7	...	26.6
$c^d c^r$ 4,3	4	34.3 ± .81	.....	....	...	15.2	15.0	96.4	95.5	47.8
$c^d c^a$ 4	13	29.5 ± .45	.....	....	...	13.1	...	69.8	...	34.1
3	10	28.2 ± .51	.....	....	...	12.5	12.4	63.7	63.2	31.1
2	9	25.0 ± .54	.....	....	...	11.1	...	50.4	...	24.4
1	3	20.8 ± .94	.....	....	...	9.2	...	36.3	...	17.4
$c^r c^r$ 4,3(2)	27	34.5 ± .31	.....	....	...	15.3	14.9	98.0	95.0	48.5
$c^r c^a$ 4	4	31.2 ± .81	.....	....	...	13.8	...	78.5	...	38.6
3	7	28.8 ± .61	.....	....	...	12.7	12.5	66.5	63.9	32.5
2	4	24.5 ± .81	.....	....	...	10.8	...	48.6	...	23.5
1	1	24.3 ± 1.62	.....	....	...	10.7	...	47.9	...	23.1
$c^d c^a$ / $c^r c^a$ 4	17	29.9 ± .31	29.3 ± .54	+ .6	29.7	13.1	...	70.8	...	34.6
3	17	28.5 ± .39	30.0 ± .71	−1.5	28.8	12.7	12.5	66.5	63.6	32.5
2	13	24.9 ± .45	23.6 ± .83	+1.3	24.5	10.8	...	48.6	...	23.5
1	4	21.4 ± .81	23.7 ± 1.58	−2.3	21.9	9.7	...	39.7	...	19.1

TABLE 9

Mean indices for various genotypes of pale sepia and derived estimates as in Table 6

Pale sepia class		(*Bpp*) No.	Reflectionmeter indices (1) Individuals	(2) Means	Diff.	Av. *I*	Visual grades From *I*	Visual grades 1949	Pigment percent black From *I*	Pigment percent black 1949
C FF	4,3,2	60	23.7 ± .25	.....	....	...	10.9	9.7	22.1	17.1
$c^k c^k$	4,3	26	18.4 ± .37	.....	....	...	8.5	9.0	14.2	15.1
$c^k c^d$	4,3	4	19.7 ± .95	.....	....	...	9.1	7.4	15.9	10.9
$c^k c^r$	4,3	13	13.7 ± .53	.....	....	...	6.3	6.7	9.0	9.1
$c^k c^a$	4,3	11	13.5 ± .57	.....	....	...	6.2	5.2	8.8	6.2
$c^d c^d$	4	6	15.9 ± .78	16.1 ± .75	− .2	16.0	7.4	...	11.3	...
	3	7	13.4 ± .72	12.9 ± .72	+ .5	13.2	6.1	4.7	8.5	5.3
	2	17	13.0 ± .46	12.9 ± .45	+ .1	12.9	5.9	...	8.2	...
	1	11	11.1 ± .57	11.5 ± .55	− .4	11.3	5.2	...	6.8	...
$c^d c^a$	4	19	8.9 ± .44	8.6 ± .42	+ .3	8.7	4.0	...	4.8	...
	3	16	8.1 ± .47	7.9 ± .47	+ .2	8.0	3.7	2.7	4.3	2.4
	2	22	8.1 ± .40	8.0 ± .40	+ .1	8.0	3.7	...	4.3	...
	1	13	6.1 ± .53	6.7 ± .51	− .6	6.4	2.9	...	3.3	...
$c^r c^r$	4,3,2	41	7.7 ± .30	.....	....	...	3.5	2.4	4.1	2.0
$c^r c^a$	4,3,2	17	2.8 ± .46	.....	....	...	1.3	0.7	1.3	0.5
C Ff		67	20.4 ± .41	.....	....	...	9.4	7.7	16.9	11.4

TABLE 10

Mean indices for various genotypes of pale brown and derived estimates as in Table 6

Pale brown class		(*bbpp*) No.	Reflectionmeter indices (1) Individuals	(2) Means	Diff.	Av. *I*	Visual grades From *I*	Visual grades 1949	Pigment percent brown From *I*	Pigment percent brown 1949
C		38	19.9 ± .36	.....	....	...	8.8	8.0	33.5	27.6
$c^k c^k$		1	16.4 ± 1.77	.....	....	...	7.3	7.2	24.6	22.6
$c^k c^a$		1	13.1 ± 1.77	.....	....	...	5.8	5.2	17.6	13.1
$c^d c^d$		23	16.8 ± .38	.....	....	...	7.4	5.4	25.5	14.2
$c^d c^r$		10	12.3 ± .56	.....	....	...	5.4	3.7	16.1	7.3
$c^d c^a$	4	26	12.7 ± .35	12.8 ± .36	− .1	12.7	5.6	...	16.9	...
	3	12	10.7 ± .51	10.1 ± .54	+ .6	10.4	4.6	2.7	12.9	4.0
	2	2	6.4 ± 1.25	8.5 ± 1.28	−2.1	7.4	3.3	...	8.4	...
$c^r c^r$		23	6.1 ± .37	.....	....	...	2.7	2.7	6.6	4.1
$c^r c^a$		4	2.9 ± .89	.....	....	...	1.3	1.0	2.9	0.9
C Ff		62	18.3 ± .34	.....	....	...	8.1	7.1	29.2	22.3
*EC*ff*(B,bb)*		122	5.0 ± .16	.....	....	...	2.2	2.3	5.2	6.2

classes were not distinguished. Table 11 shows the mean visual grade (G) of the animals for each reflectionmeter index, to the nearest unit, for the various colors. Thus the 90 reflectionmeter indices for yellow that fell in the range 9.5 to 10.4 had an average visual grade of 4.98.

There is a qualification in that 0.60 is added to visual yellow grades 9, 10 and 11. It was noted in a previous paper (WRIGHT 1949) that the gap between visual grades 8 and 9 was too great, probably because at the time the standards were set up, grades 8 (unusually intense *eec^{d}c^{a}FF*) and 9 (unusually dilute *eeCFF*) were rare. It was found that in order to establish a reasonably good linear relation between a simple function of the colorimetric values and the visual grades it was necessary to add at least 0.25 to grades 9, 10 and 11. The data were compatible with a larger correction by altering the value of an arbitrary constant.

TABLE 11

Mean visual grades (G) *of yellows, browns and sepias with the indicated reflectionmeter indices,* I

	Yellow		Pale brown		Pale sepia			Dark brown		Dark sepia	
I	No.	*G*	No.	*G*	No.	*G*	*I*	No.	*G*	No.	*G*
31	..	....	..	....	2	11.50	47	..	....	..	....
30	..	....	..	....	..	....	46	..	....	..	....
29	..	....	..	....	4	11.50	45	..	....	1	21.00
28	..	....	..	....	2	11.50	44	..	....	5	21.00
27	..	....	..	....	3	11.00	43	..	....	26	20.88
26	4	10.85	..	....	9	11.33	42	..	....	48	20.69
25	4	10.60	..	....	19	11.26	41	..	....	75	20.55
24	18	10.71	1	9.00	17	10.47	40	1	17.00	71	20.10
23	35	10.57	6	8.00	19	10.16	39	1	17.00	55	19.27
22	43	10.53	15	8.31	14	10.07	38	10	16.30	38	18.76
21	30	10.40	11	8.73	25	9.84	37	24	16.46	29	17.66
20	17	10.21	10	8.30	18	9.11	36	42	15.60	41	17.22
19	19	9.05	19	8.16	25	8.96	35	70	15.36	45	16.26
18	15	8.31	18	8.06	20	9.00	34	50	14.96	47	15.68
17	33	7.41	17	7.76	11	8.82	33	40	14.82	34	15.41
16	28	7.16	15	7.73	14	7.64	32	29	14.38	36	15.00
15	63	6.81	13	7.23	16	7.19	31	24	13.67	32	13.87
14	85	6.78	11	7.27	22	6.32	30	18	13.27	31	14.06
13	117	6.54	19	6.42	18	6.00	29	17	13.29	30	13.03
12	112	6.19	17	5.57	14	5.28	28	13	13.08	36	12.69
11	85	5.80	6	5.17	18	5.05	27	9	13.11	25	12.04
10	90	4.98	8	4.87	13	4.62	26	6	12.00	21	12.05
9	74	4.45	3	4.00	26	3.42	25	5	11.60	21	11.81
8	79	4.11	3	3.33	24	3.29	24	5	11.20	25	11.44
7	79	3.61	2	3.00	27	2.93	23	2	11.00	8	11.12
6	51	3.52	13	2.46	24	2.67	22	1	10.00	8	10.75
5	62	2.73	4	1.75	8	2.75	21	1	11.00	3	10.00
4	56	2.52	4	1.75	8	1.62	20	1	10.00	4	9.15
3	47	2.26	2	1.00	2	1.00	19	..	....	1	13.00
2	22	1.68	1	1.00	5	1.00	18	..	....	2	10.00
1	9	1.44	..	....	1	1.00	17	..	....	..	....

A reasonably good linear relation is found in plotting the mean visual grades of dilute yellows against reflectionmeter indices, but the mean grade for intense yellows (*CFF*) can only be made to fall on a continuation of this line by adding about 0.6 to visual grades 9, 10 and 11 (Figure 1). The regression equation comes out:

$$G_Y = .430674I + .7606 \qquad (0<I<26)$$

It may be seen that the regression line does not pass through visual grade 0 for index 0. It appears that there was very little difference between white and yellow grade 1. It is probable that grade 1 was usually applied to animals that showed traces of yellow on the cheeks but not on the middle of the back where the reflectionmeter reading was taken.

On plotting the mean visual grades of browns, including both pink-eyed and dark-eyed, against reflectionmeter reading it is apparent (Figure 1) that no curve

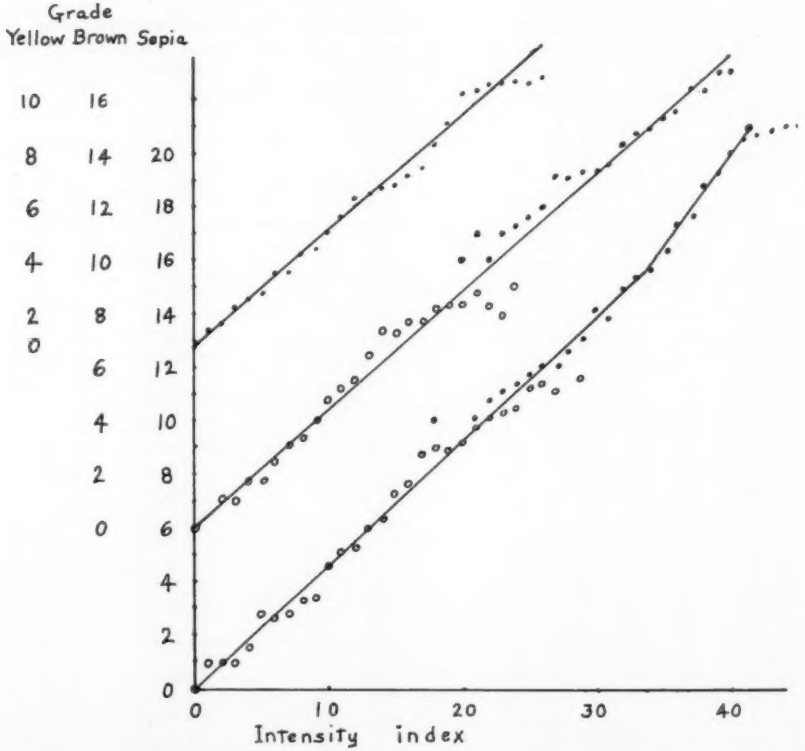

FIGURE 1.—Mean visual grades plotted against reflectionmeter indices. Data of Table 11. The fitted lines are described in the text.

can be expected to fit better than a straight line passing through the origin. There is, indeed, considerable irregularity among more intense pink-eyed browns and the more dilute dark-eyed ones. These apply, however, to only a very small proportion of those graded. The regression line is as follows.

$$G_{Br} = .44247I \qquad (0<I<40)$$

On plotting the mean visual grades of pink-eyed and dark-eyed sepias against reflectionmeter reading, it is found that there is a good linear relation up to about index value 35 apart from a few of the more intense pink-eyed sepias which do not seem to have been given sufficiently high visual grades. The regression line, which can be made to pass through the origin satisfactorily is as follows (based on values I from 1 to 35).

$$G_S = .459864\,I \qquad (0<I<33.4)$$

The more intense sepias and blacks do not, however, fall on a continuation of this line. The device used in the case of the intense yellows is not convenient here since a simple function of the colorimetric values was related linearly to the visual grades over the entire range 1 to 21 with deviations no greater than could be expected from sampling (as was also true of the browns and of the yellows on adding anything from 0.25 to 0.60 to grades 9, 10 and 11). It has seemed best to use a different line, fitted to $I = 33$ to 39 through the means for 226 blacks (mean index 41.28 mean visual grade 20.9).

$$G_S = .705913I - 8.2401 \qquad (33.4<I<45)$$

These equations make it possible to transform the indices into mean visual grades. These are shown for the various colors in Tables 6 to 10 (visual grades from I).

The previous averages of the visual grades for the c genotypes (WRIGHT 1949) are given in the next column in these tables for comparison. In the case of intense yellow (CFF) the mean is given as 10.9 instead of 10.6 because of the addition of 0.60 instead of 0.25 to grades 9, 10 and 11.

Equations were derived in a previous paper (WRIGHT and BRADDOCK 1949) for transforming visual grades into quantities of pigment (M) as indicated by colorimetry. That for yellow has been modified for reasons discussed above.

The equations for amount of melanin (M') (including melanoids found in white hair) are as follows.

Sepia	$log_{10}(M'_S + 6) = .05644\,G_S + .8768$	($M'_0 = 1.53$ is white)
Brown	$log_{10}(M'_{Br} + 6) = .05579\,G_{Br} + .8466$	($M'_0 = 1.024$ is white)
Yellow	$log_{10}(M'_Y + 20) = .09354\,G_Y + 1.4490$	($M'_0 = 13.12$ is white)

The values for visual grade (G) in terms of the reflectionmeter indices (I) can be substituted to obtain formulae for estimating M, ($M_x = M'_x - M'_0$).

Sepia	$M_S = log_{10}^{-1}$	$[.025955\, I + .8768] - 7.5300$	$0 < I < 33.4$
	$M_S = log_{10}^{-1}$	$[.03984\ I + .4117] - 7.5300$	$33.4 < I$
Brown	$M_{Br} = log_{10}^{-1}$	$[.024686\, I + .8466] - 7.02425$	
Yellow	$M_Y = log_{10}^{-1}$	$[.040285\, I + 1.5201] - 33.12$	

All of these quantities of melanin are in terms of the arbitrary standards of the 1949 paper (M'_S for blacks of grade 21, 112.9; for yellows of grade 11, 285.1). They can, however, readily be reduced to percentages of the average amounts of any desired genotype. Such percentages are presented, relative to yellows of genotype *eeCFF* in the last two columns of Table 6, to blacks (*EBCP*) in Tables 7 and 9, and to browns (*EbbCP*) in Tables 8 and 10. Those in the next to the last column are deduced from the average reflectionmeter indices while those in the last column are those given in the 1949 paper modified slightly in the case of yellow by the use of the new transformation equation.

On comparing the visual grades, estimated from the reflectionmeter indices with the 1949 averages, it is apparent that the latter is usually less than the former for 4 plus factors both with respect to yellow and sepia. The main reason is undoubtedly the high frequency which *dm* turned out to have in the general stock. Dilutes with one or even two doses of *dm* or occasionally *si* had evidently been considered as typical and only those with three doses of these factors (principally +*sidmdm*) had attracted much attention as outside the normal range of variability.

Yellow

Figure 2 and Table 6 show the indices and Figure 5 the estimated relative quantities of pigment for yellows with various *c* genotypes and numbers of plus modifiers. The former indicates best the differences to the eye.

There is no great difference between the effects of c^k and c^d. There is a suggestion on comparing $c^k c^{ra}$ with $c^d c^{ra}$ that c^k produces slightly less pigment in the presence of four modifiers but that there is somewhat less reduction in the presence of only one. The assignment of modifiers was, however, based on less adequate information than in the case of c^d, a consideration which might tend to confound the means for the different numbers of plus modifiers.

The percentage of reduction by *si* or *dm* is much greater in $c^d c^{ra}$ than in $c^d c^d$ but the absolute reduction is somewhat greater in the latter. As already noted there is no evidence that ++*dmdm* causes any reduction at all in the presence of *C*. On the other hand *sisi*++ causes about as much absolute reduction with *C* as with $c^d c^d$. The percentage reduction is much less, but this refers to the middle of the back in a spot where there was no overt silvering. On taking into account the decrease of pigment from silvering (less in $c^d c^d$ than in *C*, least in $c^d c^{ra}$) it is possible that *sisi* may produce about the same percentage reduction in all cases. In class 1 (based largely on +*sidmdm*) the percentage reduction from class 4 is clearly much the greatest in $c^d c^{ra}$ and least in *C*. There seems no doubt that *dm* at least interacts specifically with the lower *c* alleles.

C is completely dominant as far as known, $c^k c^{ra}$ produces about 53 percent as

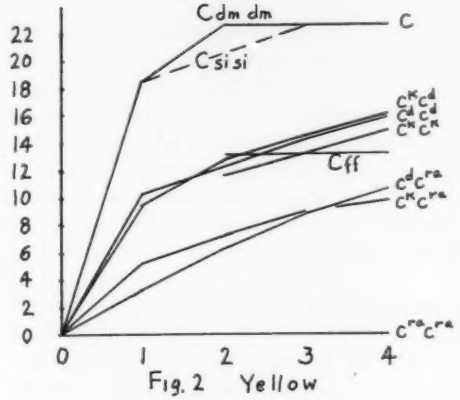

Fig. 2 Yellow

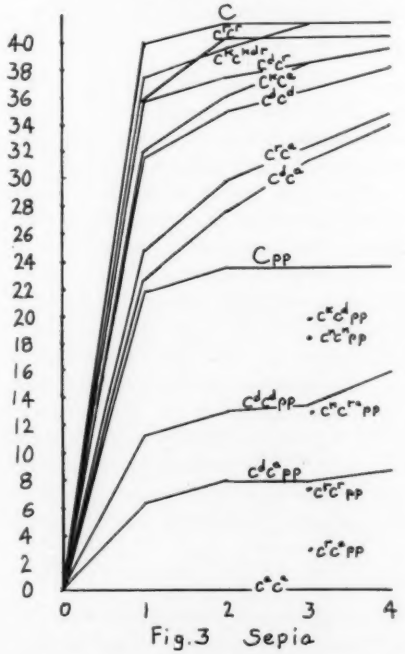

Fig. 3 Sepia

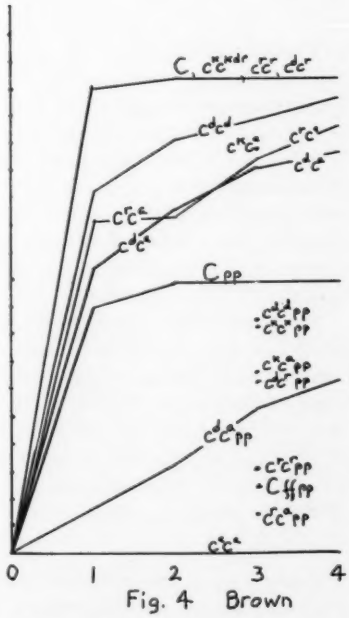

Fig. 4 Brown

FIGURES 2–4.—Mean indices for various genotypes of yellow (Figure 2), sepia (Figure 3) and brown (Figure 4) according to the number of plus modifiers of the *si* and *dm* loci.

much pigment as $c^k c^k$ (3 or 4 plus modifiers) while $c^d c^{ra}$ produces less than 48 percent as much in comparison with $c^d c^d$.

The amount of residual variability with lower *c* alleles is small (standard deviation about 0.9 on the index scale on which the range from one to four plus modifiers is 6 or 7). There was considerably more residual variability among the intense yellows (about 1.3) during the period in which the readings were taken and much more earlier when different inbred strains with the genotype *CCFF* ranged from visual grades 10 to 13. The very intense reds of grade 13 which had a somewhat brownish hue at birth are probably the same as what IBSEN (1932) has referred to as cherry reds. Crosses between strains at opposite extremes gave intermediates. There was no clear segregation in F_2 indicating multifactorial heredity.

Returning to Table 6, the rather scanty information from reflectionmeter indices on yellows that were not *FF* is summarized in the lower rows. Genes *si* and *dm* were not present as far as known. The principal points brought out are the incomplete dominance of *F* over *f*, and lack of appreciable difference between $c^k c^k$ and $c^d c^d$ when subject to slight dilution by *Ff* or extreme dilution by *ff*.

Dark sepia

Table 7 and Figures 2 and 6 give the principal results for the dark sepias. We note the absence of appreciable diminution of pigment in blacks (*C*) by *si* and *dm* except for a slight effect in the extreme combinations + *sidmdm* and *sisi* + *dm*, and of course the silvering by *sisi* which is not here considered. With lower *c* genotypes, there is, as with the yellows, a closer approach to uniform absolute amounts of diminution by *si*, *dm* combinations than to uniform percentage effects. If allowance were made for the diminution of pigment by overt silvering, there would be a closer approach to constant percentage diminution by *si* than by *dm* which as in the case of the yellows must be supposed to interact specifically with the lower *c* alleles (except in the combination *Csisidmdm*).

The dominance relations are again of interest. *C* seems to be completely dominant over all of the lower alleles. In the presence of three or four plus modifiers, $c^k c^a$ produces about 80 percent as much pigment as $c^k c^k$, $c^d c^a$ produces some 64 percent as much as $c^d c^d$ but $c^r c^a$, which produces slightly more pigment than $c^d c^a$, produces only 51 percent as much as $c^r c^r$. The dominance relations of c^r are more in line with its position among the alleles in other respects than in its effectiveness in producing sepia pigment. A similar statement could be made of c^k with respect to yellow.

Dark brown

Table 8 gives the reflectionmeter indices, estimates of visual grade and estimate of pigment for dark brown. The entries for *C* are here restricted to individuals which do not show the "dingy" type of dilution. The indices are shown in Figure 4 and relative quantities in Figure 7.

The data are fewer than desirable. They are available for varying numbers of plus alleles of *si* and *dm* only in the cases of $c^d c^d$, $c^d c^a$ and $c^r c^a$. Least square

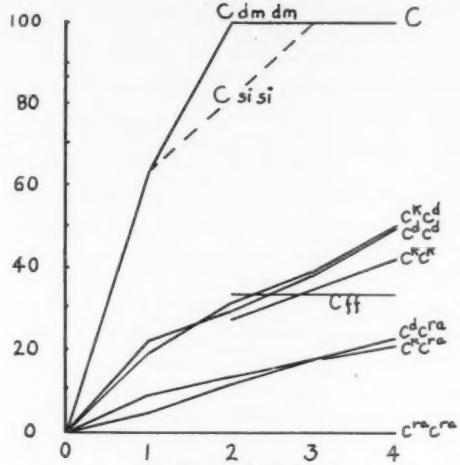

Fig. 5 Yellow

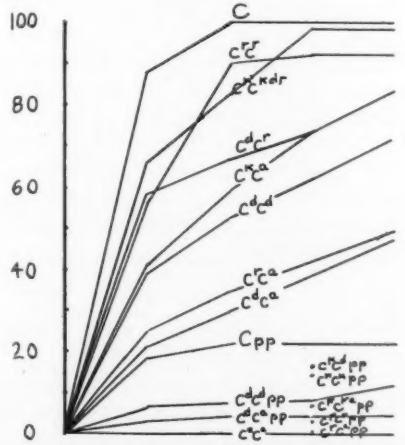

Fig. 6 Sepia

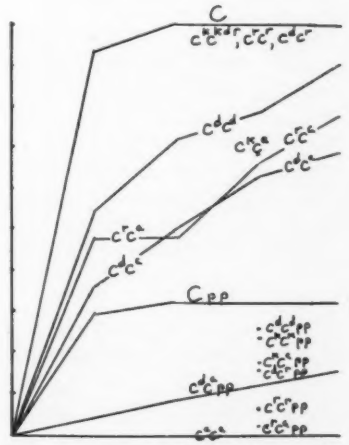

Fig. 7 Brown

FIGURES 5–7.—Estimated mean relative amounts of pigment in various genotypes of yellow (Figure 5), sepia (Figure 6) and brown (Figure 7) according to the number of plus modifiers of the *si* and *dm* loci, as deduced from the reflectionmeter indices.

estimates were made for c^dc^d and for c^dc^a and c^rc^a combined, these being closely similar. There has been no clear evidence for effects of *Si,si* or *Dm,dm* on intensity in *C* brown, although this is subject to silvering in the presence of *sisi* to about the same extent as in *C* black (WRIGHT 1959a). These loci affect the intensity of lower *c* combinations (c^dc^d, c^dc^a, c^rc^a) in much the same way in browns as in sepias.

The index for *C* brown indicates only about half as much eumelanin as in *C* black. The lower *c* genotypes, however, have much more than half as much as the corresponding sepias. As suggested previously (WRIGHT 1942) this can be accounted for by the hypothesis that there is a much more restricted limiting factor for possible amount of brown than of black so that the lower *c* alleles approach closer to the ceiling value in the former (e.g., $bbc^dc^d = 84$ percent *bbC* while $Bc^dc^d = 67$ percent *BC*). Not surprisingly, there is also a closer approach of c^dc^a to c^dc^d as the ceiling is approached (e.g., $bbc^dc^a = 79$ percent bbc^dc^d while $Bc^dc^a = 64$ percent of Bc^dc^d). These figures refer to the average estimates for three or four plus modifiers (*SiSiDmDm*). The same conclusion is indicated by comparisons with one or two plus factors in spite of some irregularities among the rather inadequately based figures for the browns. Thus with only one plus factor, $bbc^dc^d = 56$ percent *bbC* as opposed to $Bc^dc^d = 40$ percent *BC* and $bbc^dc^a = 69$ percent bbc^dc^d while $Bc^dc^a = 52$ percent Bc^dc^d. There is virtually no indication of any damping in the last case in which one dose of c^d produces just about half as much pigment as two doses.

The residual variability of the indices for brown genotypes (standard deviation 1.57 for brown genotypes excluding *C* and c^k) is somewhat greater than for sepia genotypes (1.39 also excluding *C* and c^k). Nevertheless with a range of some 7 or 8 units between the average with one dose and that with four doses of the second order modifiers *Si* and *Dm*, their residual variability is decidedly of third order and probably hardly capable of analysis. We will not consider here the dingy browns in which the combination of genes *C*, *P*, and *F* is beyond the optimum and intensity reduced in a very variable fashion.

Pale sepia

Table 9 and Figures 3 and 6 present the results from both methods of estimation. The effect of *si* and *dm* in pale sepias with c^dc^d and c^dc^a are roughly similar on a percentage basis to those in dark sepias. The fact that the effects are not much greater and that no effects on intensity have been detected in *C* pale sepia indicates that the dilution effects are specifically related to the *c* alleles rather than to amount of pigment. The markedly higher intensity of most of the genotypes in the present data as compared with the 1949 estimates is undoubtedly due in the main to selection for intensity modifiers independent of *Si, si; Dm, dm*.

Pale brown

The situation is similar in pale browns to that in pale sepias (Table 10, Figures 4 and 7). Scanty data indicate effects of *Si, si; Dm, dm* on c^dc^a pale browns. The

estimates of most genotypes are again much higher than in 1949, undoubtedly for the same reason as with the pale sepias.

Pale brownish cream

No data are available on possible effects of *Si, si; Dm, dm* on *ECppff*. Comparison of a few carrying *B* and segregating groups of which three fourths or one half are expected to carry *B*, with a considerable number known from ancestry to be *bb*, gives no indication of any difference. Notes indicate that most animals in all of those categories showed appreciable deviation from the pure pale cream that had been attributed to this genotype when first described.

SUMMARY

A large proportion of the combinations of genes *E, e; B, b; C, c^k, c^d, c^r, c^a; P, p, F, f, Si, si* and *Dm, dm* of the guinea pig have been studied with respect to the intensity of pigmentation indicated by reflectionmeter readings. A simple index has been obtained that gives results that vary linearly or nearly so with grades based on skin samples, chosen so that each grade is barely distinguishable from the preceding. These indices and visual grades are converted into estimates of relative quantities of each kind of pigment (yellow, sepia, brown) by means of previously established relations of visual grades to colorimetric determinations. The results are presented in tables.

The results for combinations of the first five of the above loci agree reasonably well with the previous results based on the visual grades. Combinations of *Si, si; Dm, dm* have roughly additive effects on intensity of lower *c* combinations, according to the number of plus factors, except that *sisidmdm* is pure white on the back where the grades were taken. Replacement of *Si* by *si*, however, brings about silvering as well as dilution. Its total effect seems to be approximately constant on a percentage basis. Replacement of *Dm* by *dm* on the other hand has little or no effect in the presence of *C* except in combination with *si*. It has maximum effect in association with lower *c* combinations.

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NEW EVIDENCE FOR THE HOMOLOGY OF THE SHORT EUCHROMATIC ELEMENTS OF THE X AND Y CHROMOSOMES OF DROSOPHILA BUSCKII WITH THE MICROCHROMOSOME OF DROSOPHILA MELANOGASTER[1]

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THE metaphase X chromosome of the mitotic and meiotic cells of *Drosophila busckii* is rod-shaped and has a secondary constriction located 1/4–1/3 its length from the proximal end. In salivary gland cells this chromosome consists of two euchromatic elements: a long distal one, and a short, proximal piece approximately 1/20th as long. Both of these elements are connected with the nucleolus. It has also been proven that the nucleolus of the polytene X is located at a site corresponding to the secondary constriction of the metaphase chromosome, and the short euchromatic polytene element corresponds to the smaller block of the metaphase chromosome that lies proximal to the secondary constriction. In salivary gland cells the centromere of the X chromosome is located at the very proximal end of the short euchromatic element, and it is probably terminally located in the metaphase chromosome as well (KRIVSHENKO 1955).

The metaphase Y chromosome is also rod-shaped, but has a primary constriction near the proximal end which divides the chromosome into two arms: a long left arm and a short, knob-like, right arm. In the salivary gland cells the Y chromosome is usually observed as a short euchromatic element that is approximately equal to the short euchromatic element of the X chromosome (SIROTINA 1938; KRIVSHENKO 1939). It has been proven that this euchromatic part of the Y chromosome is genetically active and corresponds to the right arm of the metaphase chromosome, whereas the long left arm is heterochromatic (KRIVSHENKO 1950, 1952). It was also found that XO males of this species are not viable (KRIVSHENKO 1941a, b), and that viability factors are located in the small, euchromatic right arm of the Y chromosome (KRIVSHENKO 1952).

In previous investigations of *D. busckii*, cytological and genetical evidence was obtained pointing to the homology of the short euchromatic elements of the X and Y chromosomes with each other, and indeed also with the microchromosome of *D. melanogaster* (KRIVSHENKO 1952, 1955). The basis for this conclusion lies in: 1) the observed somatic pairing of X and Y by their proximal ends in ganglion cells, and the conjugation of the short euchromatic elements of these chromosomes at their centromeric regions in salivary gland cells; 2) the presence in the short Y chromosomal element of normal allelomorphs to four different

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mutant genes of the short X chromosomal element; and, 3) the presence in the short element of the X chromosome of a mutation, Cubitus interruptus, that is characteristic of the microchromosome of *D. melanogaster*. These findings are quite sufficient to prove at least partial homology among all three of the elements. Nevertheless, these elements have been subject to very different phyletic and genetic histories, and it would be of considerable evolutionary importance to know, in the greatest detail possible, the degree to which they have retained homology as well as in what ways they have undergone evolutionary divergence.

In the present paper new X chromosomal mutations of *D. busckii* are described which are homologous to well-known microchromosomal mutations of *D. melanogaster*. Cytogenetic study of these mutations provides an explanation of why XO males in *D. busckii* are not viable, and affords a basis for some general phylogenetic conclusions.

DESCRIPTION AND CYTOGENETIC STUDY OF THE NEW MUTATIONS

The X chromosomal mutation #321 was found in the progeny of X-rayed *D. busckii* males of a wild strain from the population of Columbia, Mo. (8–10, 1949). Phenotypically characteristic of the mutant males is a fusion of the medial (L-3) and cubital (L-4) veins of the wings in their basal parts up to the region of the I-st crossvein, although this is occasionally not complete. The ocellar region of the head is smooth, and the anterior ocellus and ocellar bristles are always absent; though the posterior ocelli are usually present, they are strongly reduced. The dark spot characteristic of the ocellar region is completely absent. At 23–25°C variations are insignificant in the modification of vein structure and of the ocellar region, the morphological peculiarities of the mutant are distinctly expressed, and the viability and fertility of males are quite high. In heterozygous females wing vein structure is usually normal, though in some crosses the basal parts of the medial and cubital veins are noticeably closer to each other. The dark ocellar spot is considerably diminished, and very often one or both ocellar bristles are absent. Females homozygous for this mutation are not viable.

Cytological analysis shows a small inversion (microinversion) located at the proximal end of the salivary gland X chromosome. The left break of this inversion occurred at the very beginning of the 19th section, corresponding to the region where the nucleolus organizer is located. The right break occurred at the beginning of the 20th section, that is, in the most distal part of the short euchromatic element (see map of the X of *D. busckii* in KRIVSHENKO 1955).

Thus this semidominant, morphologically complex X chromosomal mutation is connected with a structural aberration. Whether it is caused by one gene with a pleiotropic effect, by two adjacent genes, or by a position effect brought about by the aberration is not known. However, two mutations are known in *D. melanogaster* in which a change in vein structure occurs that is very similar to that of the mutation described here. These mutations are fused and Cell. The first is X chromosomal, the second microchromosomal (BRIDGES and BREHME 1944). Now it is possible to suppose that either fused or Cell is homologous to the mu-

tation of *D. busckii;* but which one? Such an ambiguity very often arises in comparative genetical studies of species that do not cross with each other, and may present great difficulty in establishing the homologies of phenotypically similar mutations. Nevertheless, it is sometimes possible to find a convincing answer by taking into consideration the genetic surroundings of the locus under question.

Since the mutation described here is associated with an inversion, it is likely that the mutant factor is located near one of the breaks of the inversion. Should there be two independent factors, then one of them may be located at one break, the other at the other break. To check this hypothesis and to localize the responsible factor of this mutation, two experiments were performed in which special lines of flies were crossed.

In the first of the experiments *Ce/Bly* females were crossed with *Sms* males. In females of this cross one X chromosome carries the mutation under study, here designated as *Ce*. The other X chromosome is marked by the dominant mutation Blondy (*Bly*) connected with an inversion of the proximal half of the salivary gland X chromosome. Blondy flies lack pigmented abdominal bands and have a yellowish body. The males of this cross have a dominant X chromosomal mutation, Small spots (916) (the abdominal pigmented bands are very reduced), the factor for which is located in a small X chromosomal part inserted into IIIL. The breaks in the X chromosome that gave rise to the small inserted part occurred to the left at the end of section 17, and to the right somewhere in the center of section 19. Thus the first break is located considerably to the left, the second a little to the right of the distal break of the inversion in the mutant strain. Because the X chromosomal part carrying the factor Small spots is inserted into IIIL, Small spots is inherited as a dominant autosomal factor and is independent of the normal X chromosome.

The results of this cross are shown in Table 1. Since the mutation Blondy is epistatic to the mutation Small spots, six phenotypically different classes of flies appear in the progeny.

Of these flies, the males of class *Ce;Sms* are of special interest. These males

TABLE 1

Determination of the location of gene Ce *by means of the cross*

$$♀♀\ \frac{Ce}{Bly}\ \frac{+}{+} \times ♂♂\ \frac{+}{-}\ \frac{Sms}{+}:$$

Phenotypic classes	Number of flies
♀♀ *Ce; Sms*	91
♀♀ *Ce*	125
♀♀ *Bly*	198
♂♂ *Ce*	91
♂♂ *Ce; Sms*	83
♂♂ *Bly*	141
Total	729

carry the X chromosome of the investigated mutant strain #321, as well as the X chromosomal duplication phenotypically manifested by the characteristic Small spots. In spite of the fact that the *Sms* duplication covers the region of the distal break of the inversion in the *Ce* mutant line, the *Ce;Sms* males have the distinctive phenotypic complex of males of the *Ce* line expressed without any changes. Thus the appearance of these males in the progeny of this cross indicates that the hereditary factor causing the *Ce* phenotype is located neither at the region, nor close to the region, of the leftmost break in the X chromosome of the mutant line #321.

In the other experiment *Ce/Bly* females of the same origin as in the first experiment, were crossed with *mo/Ri* males. The males of this cross have complex X and Y structural changes of independent origin; that is, their chromosomal complement consists of two aberrant haploid sets of euploid constitution. The X chromosomal recessive mutation mosaic (*mo*, 188) (black spotted eyes and other effects) is connected with an aberration that involves X and IIIR. The X chromosome was broken in two regions, namely at the very beginning of the 2nd section and in the distal half of the 19th section (but to the left of the rightmost break of the inversion associated with the mutant *Ce*). The break in IIIR is located at approximately one fifth the length from the distal end. The long, middle part of the X chromosome, to the proximal end of which the short, distal portion of the X was joined, was translocated in an inverted position onto the proximal part of IIIR. The distal part of IIIR united with the proximal or centromeric part of the X chromosome. As a result of this last union, an aberrant short X chromosome was formed of which the X chromosomal part consists only of the basal part of the 19th (a heterochromatic, nucleolar region) and the whole of the 20th section (a short, euchromatic element). The dominant mutation Radius incompletus (*Ri*) is connected with a complex aberration involving Y and IIR. In its inheritance this autosomal mutation cannot be separated from the Y chromosome. The results of this cross are given in Table 2.

The first four of six classes of flies obtained in the progeny of this cross are regular, having derived from segregation that produce haploid euploid complements from the aberrant sets of chromosomes present in the parental males. However, the two last classes, namely ♂ ♂ *Ce*/? and ♂ ♂ *Bly*/?, are males of an exceptional type. The striking peculiarity of these males consists in the absence of Radius incompletus from their phenotype. Because Radius incompletus is inseparable in its inheritance from the Y chromosome, its absence shows the absence of the Y chromosome.

The occurrence of 143 XO males would at first seem absolutely incompatible with earlier well-established observations and experimental data. Our studies of primary and secondary nondisjunction of the sex chromosomes in both males and females of *D. busckii* have shown that adult exceptional XO males are not produced in this species. Although 73 exceptional females have been found among 85469 flies obtained from special crosses carried out for the study of primary nondisjunction in males and females, *no* exceptional males have been found

TABLE 2

Determination of the location of gene Ce *by means of the cross*

$$\text{♀♀}\ \frac{\text{Ce}}{\text{Bly}}\ \frac{+}{+}\ \frac{+}{+} \times \text{♂♂}\ \frac{\text{mo}}{\text{Ri}}\ \frac{+}{*}\ \frac{*}{+}\ :$$

Phenotypic classes	Number of flies
♀♀ type Ce (heteroz.)	154
♀♀ type Bly	216
♂♂ Ce/Ri	38
♂♂ Bly/Ri	137
♂♂ Ce/?	58
♂♂ Bly/?	85
Total	688

* The asterisks indicate the autosomes involved in the translocations that mark X(*mo*) and Y(*Ri*).

(KRIVSHENKO 1941a,b,c). On the basis of these results the conclusion was drawn that the Y chromosome of *D. busckii* is a genetically active chromosomal element necessary for the normal development of males. This conclusion has since been repeatedly confirmed by numerous observations and experiments (unpublished data).

Nevertheless, the occurrence of males without a Y chromosome in the experiment described above is indubitable. Evidently in this experiment, therefore, specific conditions exist that permit the appearance of such males. These conditions are expressed in the phenotype of males of *Ce*/? class, and cytologically in males of both these exceptional classes.

Although the males of *Ce*/? class have the maternal X chromosome of the mutant *Ce* strain, the change in wing vein structure is not expressed at all. The phenotypic changes in head characteristics are expressed by the reduction of the dark ocellar spot and by the absence of one or two ocellar bristles. The phenotypical expression of the *Ce* characteristics in *Ce*/? males is, therefore, exactly similar to that of ordinary females heterozygous for the new mutation. Such a modified expression of the phenotype in *Ce* mutant males had never been observed before nor do the *Ce*/*Ri* males of this experiment show the slightest reduction in the Ce phenotype.

The similarity in phenotype of the *Ce*/? males with that of heterozygous females gives reason to suppose that these males are also heterozygous for the factor (or factors) determining this X chromosomal mutation. This may be caused by the presence of a duplication for the region on which *Ce* is localized. The paternal short X chromosome of the mutant mosaic, consisting of the small distal part of IIIR and of the short proximal part of the X chromosome, is exactly such a duplication. It undoubtedly represents the chromosomal part containing the genetic factor (or factors) causing heterozygosity for *Ce* and compensating for the viability factors normally present in the Y chromosome.

There is no doubt that the same conditions are also responsible for the appear-

ance of the Blondy males of the *Bly*/? class. In them, however, heterozygosity for the proximal part of the X chromosome is not expressed because of the absence of an appropriate marker in this part of the chromosome.

A parallel cytological analysis of the salivary and ganglion cells of male progeny in the given cross completely corroborates the above conclusions. Thus the males of both exceptional classes mentioned above have the short paternal X-IIIR translocation instead of a Y chromosome; hence, these exceptional males are X/dp-X, and not truly XO.

Thus, from the data of this cross, it follows that the hereditary factor of the new mutation *Ce* is located wholly in the region of the proximal break of the inversion. It is therefore located at the beginning of the 20th section, that is, in the distal part of the short, proximal, euchromatic element of the polytene X chromosome. Because the factor Cubitus interruptus is also located in the same part of the X chromosome, and of course has its homologue in the microchromosome of *D. melanogaster*, the mutation described here undoubtedly is a homologue of the phenotypically similar microchromosomal mutation Cell.

The other X chromosomal mutations #960 (10–7, 1958) and #969 (10–9, 1958) were found in the progeny of X-rayed males of a wild strain from the population at Princeton, N. J. The morphological peculiarities of the mutant males of both stocks are expressed by (1) a reduction in the abdominal bristles (of both sternites and tergites), the degree of reduction varying from a complete disappearance to a differential reduction in the size of these bristles; (2) in the absence of one or both ocellar bristles, and some of the orbital bristles. The viability and the fertility of the mutant males are high. Neither mutation produces viable females when homozygous. Compound females having both mutant X chromosomes (that is, 960–969) are also unviable. However, heterozygous females are phenotypically normal and have both high viability and fertility. Cytological analysis of the salivary gland chromosomes disclosed no macrochromosomal aberrations. Thus we have here recessive mutations, very likely homologous and connected with a homologous recessive lethal.

No mutations among the recorded X chromosomal mutations of *D. melanogaster* (BRIDGES and BREHME 1944) are morphologically similar to those just described for *D. busckii*. Of the autosomal mutations which are phenotypically expressed by the reduction of abdominal bristles, the microchromosomal mutation shaven has the greatest resemblance to them. Nevertheless, morphological similarity alone is, of course, insufficient to draw conclusions about homology. In this case, as for Cell, the important criterion must be the positions of the loci of the mutations under investigation to those of other mutations which are already known and typical for the given chromosome or chromosomal part.

Several different crosses were carried out to locate the hereditary factors of the mutations described here, one of which was most demonstrative and its results are given below. In this cross *sv*/*Bly* females were crossed with *mo*/*Ri* males. Structurally this cross is similar to that used in the second experiment localizing the mutant Cell. The only difference is that the factor of mutation #969 is locat-

ed in one of the X chromosomes of the females, and is designated as *sv;* all else is the same as in the Cell experiment.

The structural peculiarities of the parents again determined the appearance of six phenotypic classes of flies as shown in Table 3.

TABLE 3

Determination of the location of the gene sv *by means of the cross*

$$♀♀ \frac{sv \; + \; +}{Bly \; + \; +} \times ♂♂ \frac{mo \; + \; *}{Ri \; * \; +}$$

Phenotypic classes	Number of flies
♀♀ type normal	106
♀♀ type Bly	100
♂♂ sv/Ri	80
♂♂ Bly/Ri	81
♂♂ type normal	94
♂♂ type Bly/?	59
Total	520

* See Table 2.

The first four of the classes shown in Table 3 are regular and expected under ordinary conditions of chromosomal segregation (see the second experiment), but the males of the last two classes are exceptional. Their peculiarity consists in the absence of the dominant marker *Ri* which, as was indicated above, cannot be separated from the Y chromosome in its inheritance.

Since males of the XO constitution of *D. busckii* are not viable, the appearance of the exceptional classes of males is again due to the presence of a genetically adequate substitute for the Y chromosome carrying factors for male viability. Cytologically it was again proved that this substitute is the short duplication derived from the parental males which consists of the telomeric part of IIIR and the proximal part of X representing the short euchromatic element of X. Undoubtedly the genetic factors which substitute for the Y chromosomal male viability factors are located in the X chromosomal euchromatic part as is also the normal allelomorph of the hereditary factor of the mutation *sv* here investigated. The presence of this normal allelomorph explains the fact that males of the 5th class are phenotypically normal. The data for mutation #960 are completely similar.

Since the common hereditary factor of mutations #960 and #969 is located in the short euchromatic element of the X chromosome in which the mutation Cubitus interruptus is located, and since these mutations are identical and phenotypically similar to the microchromosomal mutation shaven of *D. melanogaster*, it may be concluded that they are in fact homologous with the mutation shaven. Thus both of the two new mutations of *D. busckii*, Cell and shaven, present new

genetical evidence for the homology of the short euchromatic element of the X chromosome of *D. busckii* with the microchromosome of *D. melanogaster*.

DISCUSSION

Our previous investigations proved that the Y chromosomal factors for male viability are located in the right arm, which is observed as a short euchromatic element in the salivary gland cells. When this arm or element is absent, males are not viable (KRIVSHENKO 1952). In the experiments summarized in Tables 2 and 3 exceptional males appeared in which the Y chromosome was substituted by the short euchromatic element of the X chromosome. The fact that these males were perfectly viable and without any phenotypic abnormalities shows that factors for male viability are not unique to the Y but they can be substituted by X chromosomal allelomorphs. The localization of these allelomorphs in the short euchromatic element of the X chromosome represents a new and additional genetic proof of homology of this element with the euchromatic part of the Y chromosome. Nevertheless, the X chromosomal part cannot be a complete substitute for the Y chromosome because the exceptional males obtained in these experiments are completely sterile.

Earlier data had indicated the existence of an homology of the X and Y chromosomes with each other as well as homology of X with the IV chromosome of *D. melanogaster*, allowing these elements to be placed in an order, Y-X-IV, suggesting their genetic relationship. The lines connecting the adjacent elements indicate direct evidences of relationship between these elements. It was not possible to draw such lines of homology between Y and IV because of the absence of any direct confirmatory data. However, it is now possible to demonstrate this homology through an analysis of the genetic behavior of the mutants Cell, shaven and Cubitus interruptus.

The behavior of both the semidominant factor Cell and the recessive factor shaven differs in males and in heterozygous females in a manner typical for X chromosomal mutations that do not have normal allelomorphs in the Y chromosome. Even though the homozygous females are not viable and hence we do not have a complete picture of the phenotypic expression of these mutations, nevertheless, the phenotypic expression in hemizygous individuals gives sufficient ground to conclude that the process of dosage compensation is already complete. As a result of dosage compensation the original autosomal factors now express themselves in a typically X chromosomal manner. It is hard to say what has happened to the Y chromosomal allelomorphs of the factors for the mutations Cell and shaven if, in fact, a part homologous to that of X which bears *Ce* and *sv* is present in the short euchromatic element of the Y chromosome. The immediate connection of these mutations with lethals, of which normal allelomorphs are present in the Y chromosomal element, gives some reason to assume that such a part is still present.

The dominant mutation Cubitus interruptus (585) behaves differently. The phenotypical expression of *Ci* is the same both in mutant males (possessing an

aberrant X and a normal Y) and in heterozygous females (possessing an aberrant and a normal X chromosome). In mutant males in which the Y chromosome is substituted by short X chromosomal duplication from the stock of mosaic (188) (as in exceptional males of second and third experiments), the phenotypic expression is also the same.

As was indicated in my previous work (KRIVSHENKO 1955), the occurrence of the mutation Cubitus interruptus was connected with a complicated series of chromosomal aberrations that involved breaks in X, IIR, IIIL and IIIR. As a result of the rearrangement of the parts of the chromosomes, the aberrant X chromosome fragment bearing this mutation is now a short (dot-like) chromosome consisting of the telomere part of IIIR and the proximal part of the short euchromatic element of the X chromosome; it is in the latter element in which the locus of Cubitus interruptus is located. Due to the small size of this chromosome and its independent behavior at the reduction division, it is easily extracted from the aberrant euploid set and may then be introduced as a duplication into chromosomal complements of flies of wholly different chromosomal constitutions. It is, therefore, possible to study the phenotypical expression of the factor Cubitus interruptus in different genetic environments. In this way it was established that males and females which, in addition to their normal chromosomal complements, have the short X duplication carrying *Ci* are phenotypically identical with the flies of the original, complexly rearranged *Ci* stock.

Thus the phenotypic expression of the mutation Cubitus interruptus is the same in both females and males of different chromosomal structure and does not seem to depend upon the number of the normal allelomorphs that are possibly present in their sex chromosomes (at least in the X). Whether this is due to an absolute dominance of *Ci*, or to the presence of a normal allelomorph in the Y chromosome creating equivalent genetic conditions in both sexes, cannot be decided for it is impossible at present to obtain males of XO structure.

Nevertheless, the genetical behavior of this mutation indicates that it should be placed in the group of so-called neomorphic mutations, for the phenotypical expression basically depends upon the gene dosage (MULLER 1932a). The mutation Cubitus interruptus displays the same peculiarities in males as well as females possessing a double dose of the factor (in presence of a normal X or Y), both sexes exhibiting a very strong, peculiar, and identical expression of the phenotype.

The genetic nature of neomorphic mutations is not always clear, but it is certain that many of them represent some form of position effect. The possibility that the mutation Cubitus interruptus of *D. busckii* also represents a position effect cannot be excluded. However, independently of the nature of this mutation, it is important to know whether in the short euchromatic element of the Y chromosome there is a part homologous to that of the X chromosome in which the mutation Cubitus interruptus arose. This problem is very difficult and an experimental solution seems impossible. But the fact that this mutation is connected with a recessive lethal for which the normal allelomorph is present in the short

euchromatic element of the Y chromosome gives a reasonable basis for concluding that the short euchromatic element of Y does possess a section homologous with that of X in the *Ci* region.

Of course, it is impossible to draw a definite conclusion as to whether the homologous part of the Y chromosome still preserves genetic potentialities similar to those of the corresponding part of the short euchromatic element of the X chromosome, and is in fact still capable of producing a change of the Cubitus interruptus type. Undoubtedly processes of inactivation or losses of genic material (as evidently has occurred in the cases of the mutations Cell and shaven), as well, perhaps, as processes of genic redifferentiation (BERG 1937a,b) leading to changes in the genetic material have taken place. Proof of this last process can be seen in the appearance of factors for male fertility in the short euchromatic element of the Y chromosome (KRIVSHENKO 1952). These factors do not have normal allelomorphs in the short euchromatic element of the X chromosome.

It may be concluded that the factors for viability in the Y chromosome that are homologous to corresponding factors in the X chromosome, and which are immediately connected with the hereditary factors of the mutations Cell, shaven and Cubitus interruptus, represent an indirect proof of the homology of a Y chromosomal part of *D. busckii* with the microchromosome of *D. melanogaster*. We may at last represent the pattern of homology not in the form of a line (Y-X-IV) but in the form of a triangle at the apexes of which the chromosomal elements under discussion are situated (or, represented linearly, Y-X-IV-Y).

Inasmuch as homology of the short euchromatic elements of the X and Y chromosomes of *D. busckii* with the microchromosome of *D. melanogaster* has now been established, one might expect additional correspondence of the genic contents of these elements that determine morphogenetic and other functions in these two species of Drosophila. However, the presence of the viability factors in the Y chromosome which have their allelomorphs in the X chromosome do not fit this pattern. Indeed, a double dose of these factors is needed for the normal development of the male individuals of *D. busckii*. When one element (or even part of one) is absent, males do not appear, and it is likely the situation is the same for females as well. In *D. melanogaster* it is otherwise, for males and females of this species having but one microchromosome (so-called "haplo-IV" progeny) are viable, albeit poorly so, their fertility is low, and in addition they have characteristic phenotypical abnormalities (BRIDGES 1921; STURTEVANT and BEADLE 1939; and others).

Before drawing a direct homology between the short euchromatic elements of the X and Y chromosomes of *D. busckii* with the microchromosome of *D. melanogaster*, it is necessary to answer the question as to how, where, and from what the viability factors came into being which must now be present in double dose in the sex chromosomes. It is not known fully how many such factors are present in the parts of the sex chromosomes under investigation, but there are at least seven of them. It is implausible to assume that they appeared *de novo*, or as a

result of redifferentiation after microchromosomes of the *D. melanogaster* type became part of the X and Y chromosomes of *D. busckii*, for there is no crossing over between the heterochromosomes. It is quite evident that these factors are of an autosomal origin, for the fact that they are needed in a double dose, unlike normal X chromosomal factors, indicates they have not acquired dosage compensation. It is accordingly reasonable to assume that the ancestral form of the short euchromatic elements did in fact carry and supply these factors.

It is likewise possible to suppose that originally the microchromosome of *D. melanogaster* also possessed these factors but that in the evolution of this species they were either lost or underwent definite changes. The appearance of haplo-IV individuals would then be explained simply by the small size of this chromosome. But the euchromatic part of this chromosome in the salivary gland cells has a comparatively large size comprising more than 135 bands (SLIZYNSKI 1944) and autosomal deficiencies comparable in size and extent to the microchromosome are generally lethal. It is, therefore, hardly reasonable to suppose that this chromosome has less significance in the development of an individual as compared with comparable parts of other autosomal chromosomes. Indeed this cannot be correct if only for the reason that the microchromosome is present in the chromosomal complements of all species of Drosophila that have been cytologically investigated to date, and can be made out in salivary gland preparations.

It is well known that structural changes of the karyotype, as well as changes in the genic composition of separate chromosomal elements, represent continuous processes in the evolution of organisms. It is also known that conditions which determine at least some of these processes are different for sex and autosomal chromosomes. This viability of haplo-IV individuals may be explained by assuming that there was a time when the microchromosome was subjected to the same conditions which determined the nature of the X chromosomal factors. In other words, the microchromosome of *D. melanogaster* was in the past evidently a part of the X chromosome and dosage compensation was brought about for many hereditary factors that are located in it. Some of the synaptical peculiarities of this chromosome expressed by non-random segregation seem to be in accordance with this assumption (STURTEVANT 1934, 1936; GERSHENSON 1941; LINDSLEY and SANDLER 1956).

Evidently such an hypothesized original form, with the IV chromosome element as a part of the X, was ancestral to *D. melanogaster* as well as other closely related species (STURTEVANT 1946). *D. ananassae*, it seems, may represent one of the descendant species. But the line of separation of the proximal end which gave origin to the IV chromosome of this species must have passed to the left of the nucleolus organizer. As a result the IV chromosome of *D. ananassae* includes both the microchromosomal material and the heterochromatic part of the X chromosome in which the nucleolus organizer and the bobbed locus is located (KIKKAWA 1938; KAUFMANN 1937).

If this assumption is valid, the conclusion follows that the present condition of the microchromosome of *D. melanogaster* is not primary, nor is that of the

short euchromatic elements of the X and Y chromosomes of *D. busckii*. For these reasons we think it is necessary to look for the precursor of the short euchromatic elements of *D. busckii* in the microchromosomal elements of another species of Drosophila, in which they possess greater genetic potentialities than the microchromosome of *D. melanogaster*, or, put otherwise, in which the ancestral element had not departed so far from a typically autosomal nature.

The inclusion of the autosomal elements into the sex chromosomes is a widespread phenomenon in different groups of organisms (WHITE 1954) and, undoubtedly, is one of the general processes of karyotypic change. The species of Drosophila represent no exception in this respect (PATTERSON and STONE 1952). The mechanism of this process in all observed cases is similar and comparatively simple, namely translocation or so-called "centric fusion" of whole chromosomal elements. As a result, a complicated reconstruction of the genic balance of the newly formed karyotype may occur in many cases. Without question this reconstruction is a protracted process with a definite sequence.

Thus, as the results of this study of *D. busckii* suggest, the gene complex of the autosomal element that is now a part of the X chromosome still preserves at least some of its morphogenetic functions, despite the fact that some of the components of this complex now show X chromosomal peculiarities. The homologue, which has become part of the Y chromosome, no longer appears to possess normal allelomorphs to the morphogenetic factors located in the X chromosome. Nevertheless, comparatively numerous homologous viability factors are still preserved in both these elements and their autosomal nature is unchanged. Thus, whereas in the X chromosomal element processes of adaptation to new conditions took place, in the Y chromosomal element different processes occurred which led to the loss of the normal allelomorphs of these factors.

The origin of the Y chromosome in Drosophila as well as in other organisms is the subject of many discussions in the genetic literature and represents one of the more enigmatic problems of biology. MULLER (1914, 1918, 1932a,b) and NEUHAUS (1938, 1939) suggest that the reduction of this element to a nearly inert chromosome is due to inactivation, or simply to the loss of genes, of a formerly genetically active element initially homologous to the X chromosome. The condition which favored the accumulation of the products of inactivation was an absence of crossing over between X and Y. The loss of morphogenetic factors in the Y chromosome of *D. busckii* is consistent with such an hypothesis, and, furthermore, represents the first experimental evidence of the correctness of this hypothesis. But in the evolution of this Y chromosomal element there occurred not only processes of inactivation and loss, but undoubtedly also processes of redifferentiation leading to the appearance of the factors for male fertility.

SUMMARY

1. Two new X chromosomal mutations of *D. busckii* homologous to the microchromosomal mutations Cell and shaven of *D. melanogaster* are described. The fact that the genes involved in these mutations are located in the short euchro-

matic element of the polytene X chromosome, close to the locus of the previously described Cubitus interruptus, serves as new proof of the homology of this chromosomal element with the microchromosome of *D. melanogaster*.

2. For the first time males of *D. buscki* were obtained that do not have a Y chromosome. Such XO males appear only when the Y chromosome is substituted by the short, proximal euchromatic element of the X chromosome. The Y chromosomal factors for male viability are, therefore, not specific to the Y but have homologs or allelomorphs in the proximal part of the X chromosome.

3. In a previous paper it was proved that the Y chromosomal factors for male viability are located in the right arm of this chromosome which is observed as a short euchromatic element in the salivary gland cells. The presence of homologous factors for viability in the short euchromatic elements of both the Y and X chromosomes provides new evidence for the homology of these chromosomal elements, and must derive from their common evolutionary origin.

4. On the basis of comparative analyses of the genetical data, some general phylogenetical conclusions are drawn.

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THE EFFECT OF LOZENGE PSEUDOALLELES ON EYE PIGMENTATION IN DROSOPHILA MELANOGASTER. III. PIGMENT DEVELOPMENT DURING PUPAL DIFFERENTIATION

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ONE method of studying the pleiotropic actions of a gene, as expressed in various phenotypic effects in the adults, is the analysis of gene effects in a multiple allelic series. One group of such mutants which has been analyzed extensively by a number of different methods is the lozenge series of pseudoalleles in *Drosophila melanogaster*. One of the phenotypic effects of the lozenge genes may be observed as abnormalities in the structure and pigmentation of the compound eyes. A series of histological studies of the lozenge pseudoalleles have shown a correlation between different phenotypic effects and a single gene action on development, the failure of the cells to differentiate normally. The structure of the adult lozenge eyes and the pupal development of the mutant eyes were analyzed histologically (CLAYTON 1952, 1954b) and it was found that the failure of the ommatidial cells to differentiate normally led to the irregularities in the adult eye structure and facet appearance. A series on pigmentation in adult lozenge eyes and the structure and pigmentation in eyes of lozenge compounds (CLAYTON 1954a, 1957, 1958) indicate that a correlation exists between the structural abnormalities of the ommatidia and the distribution of the pigment granules. In the present study, the deposition of pigment during pupal development of lozenge eyes is analyzed in an attempt to correlate more closely the effects of the lozenge gene on eye structure and pigmentation.

MATERIALS AND METHODS

Pigmentation was analyzed during pupal development for *Drosophila melanogaster* Stephenville and eight members of the lozenge series: lozenge (*lz*), lozenge glossy (lz^g), lozenge 34k (lz^{34k}), lozenge y4 (lz^{y4}), lozenge 3 (lz^3), lozenge spectacle (lz^s), lozenge spectacle-Bishop (lz^{sB}), and lozenge 36c (lz^{36c}). A less detailed analysis was made on lozenge Bar-Stone (lz^{B8}). These mutants were maintained as balanced isogenic stocks using the *ClB* inversion (OLIVER 1947).

The age of the pupae was determined from the time of egg deposition and pupation. The techniques used for obtaining developmental stages of a particular age have been described in detail in an earlier paper (CLAYTON 1954b). Pupae were collected every five hours from the time when pigment first appeared until emergence of the adults. The heads were removed from the pupae and

unstained slides were prepared by passing sectioned material rapidly through changes of xylol, alcohol, and xylol. The unstained sections were compared with stained material from the previous study on development; the descriptions of normal development in wild type and abnormal differentiation in a group of lozenge alleles may be found in an earlier paper (CLAYTON 1954b).

RESULTS

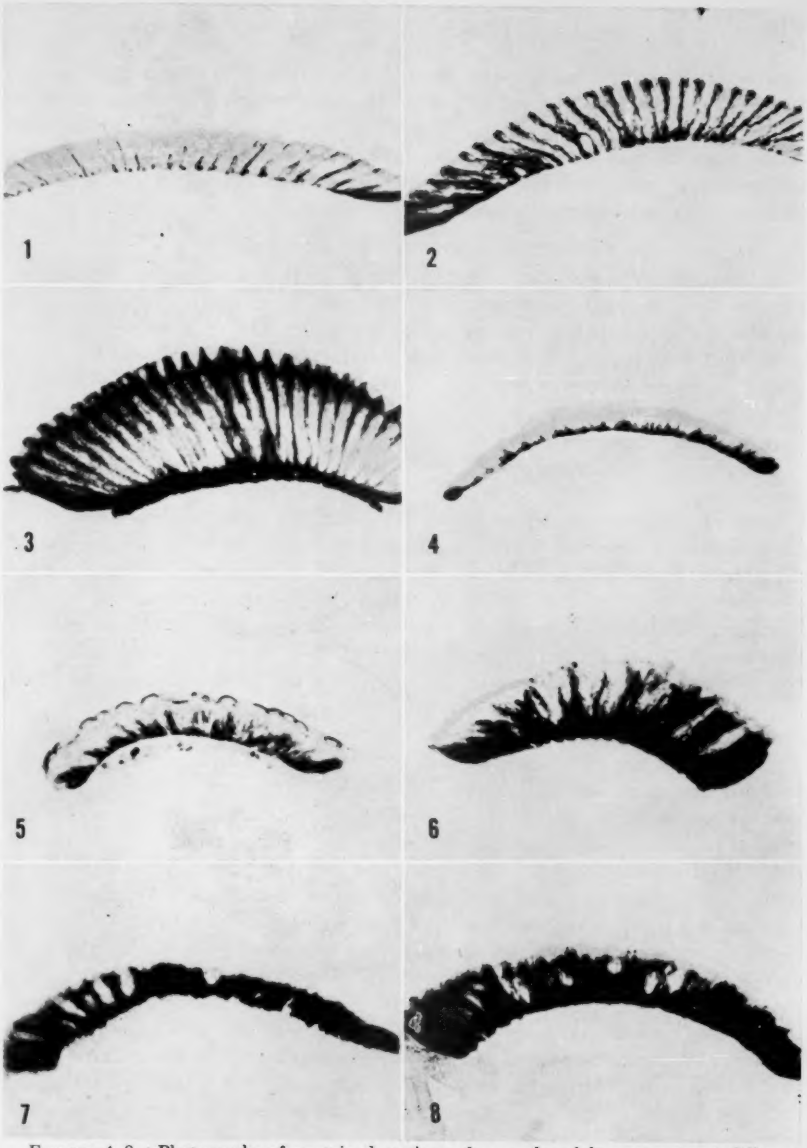
Pigment development in the wild type eye

The first indication of pigmentation in the wild type eye appears at about 160 hours (42 hours after pupation) as a slight yellowish color. The pigment seems diffuse but this appearance may be the result of the solubility of the pigment in the fixative. The ommatidia at this time have undergone slight elongation and all ommatidial cells can be observed. The pigment is concentrated in the basal portions of the secondary pigment cells and in the basal pigment cells.

At 165 hours the pigment granules are yellowish-brown. The concentration at the basement membrane is heavier and the distribution extends peripherally in the secondary pigment cells about one third of their length. No pigment could be seen in the primary pigment cells but some brown granules were present in the postretinal pigment region. By 175 hours the brown pigment granules are present throughout the length of the secondary pigment cells and are concentrated more heavily in the basal cells (Figure 1). Very little pigment was observed in the postretinal concentration; a few scattered granules are present in the primary pigment cells. The over-all color at this time is yellowish-brown, similar to the color of the adult eyes of the brown mutant. A heavier concentration of pigment in marginal regions is dull brown, lacking the yellowish appearance of the less concentrated regions.

The color at 180 hours is light reddish-brown, confined primarily to the secondary pigment cells and basal pigment cells, although some clusters of pigment have appeared in the postretinal cells. By 185 hours the mixture of red and brown granules can be seen. The color is irregular in different regions; some ommatidia are wine red in color while others are brown toward the basal region and red peripherally. The distribution of pigment in the postretinal concentration is quite distinct, with granules appearing in rounded masses in the cells and in the processes extending upward toward the basement membrane. The pigmentation in the primary pigment cells is irregular at this time.

By 190 hours pigment granules have appeared in the primary pigment cells and the color of the eye is wine-red (Figure 2). The appearance is very similar to the adult condition except for darkening of the color in the granules and completion of pigmentation in the primary pigment cells. The pseudocone cups have not formed at this time and the color in the primary pigment cells is irregular, varying from orange to red. The changes in pigmentation from 190 hours to emergence of the adults between 210 and 215 hours is primarily an increase in the amount of pigment in the granules and increased pigmentation in the primary pigment cells. In pupae of this age the separate postretinal pigment cells



FIGURES 1–8.—Photographs of unstained sections of normal and lozenge eyes at different stages of pupal differentiation. Carnoy's fixative, initial magnification × 200. FIGURE 1.—Normal, 172 hours. FIGURE 2.—Normal, 190 hours. FIGURE 3.—Normal, 210 hours. FIGURE 4.—*lz*, 170 hours. FIGURE 5.—*lz*, 190 hours. FIGURE 6.—*lz*, 205 hours. FIGURE 7.—lz^g, 195 hours. FIGURE 8.—lz^g, 210 hours.

are very distinct and more easily studied than the heavily pigmented cells of the adult eye (Figure 3). Just before emergence the structure is like the adult eye and pigmentation is similar to that of a recently emerged adult. The primary pigment cells and the distal portions of the secondary pigment cells are orange-red in color while the postretinal cells, basal cells, and the proximal portions of the secondary cells are dark red.

Pigment development in the lozenge series

In tracing the development of pigment in members of the lozenge series, eight of the pseudoalleles were studied in detail, from the first appearance of color in the pupal eye until emergence of the adults. The onset of pigmentation in the different regions of pigment concentration and a comparison of the pigmentation in normal ommatidia and abnormal cells are summarized in Figure 13. Detailed descriptions of the development of structural abnormalities were presented earlier (CLAYTON 1954).

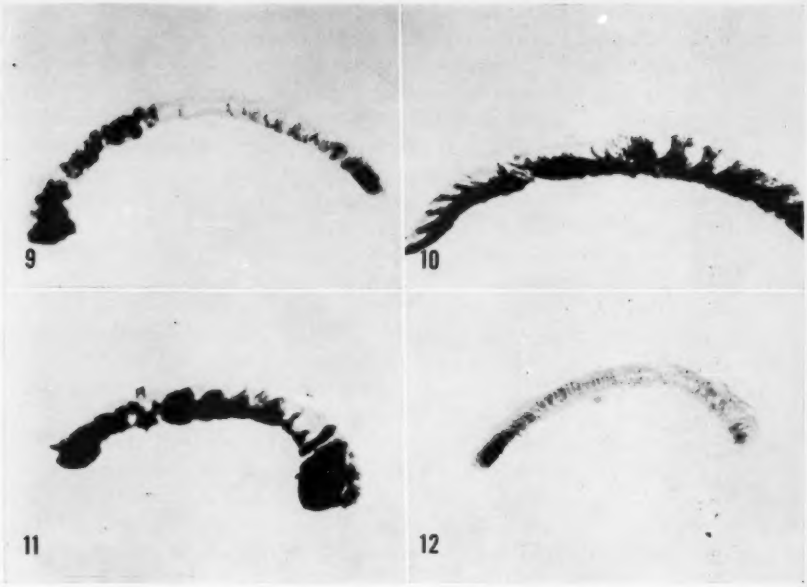
The eyes of adult lozenge males are dark red in color and closely resemble, in pigmentation, the wild type eye color. The distribution of the pigment and the amount of pigment are normal except in regions of structural abnormalities. In the pupal development of the eye, pigment is first observed in the basal region of the normal secondary pigment cells and the normal basal pigment cells. At the onset of pigmentation, after 165 hours of development, the pigment color is a diffuse light yellow. By 170 hours the pigment in the basal region of the ommatidia is yellowish-brown in color. A small amount of pigment can be seen occasionally below the basement membrane but none is associated with the abnormal retinulae in the postretina at this time. These irregular retinulae, however, occasionally disrupt the pigment of the basal and secondary cell regions (Figure 4). Individuals at 175 hours have yellowish-brown pigment granules extending peripherally about one half the length of the secondary pigment cells. No pigment is present around retinulae in the postretina. Brown pigment granules are present throughout the length of the secondary pigment cells in pupae of 180 hours. In the distal portions of these cells and in some of the basal cells the color is reddish-brown, but the over-all color is brown. A few scattered granules of pigment are present in the normal primary pigment cells at this time. At 190 hours brown pigment may be observed with abnormal cells of the postretina and in the distorted primary pigment cells (Figure 5). The eye color at this time is dark red, similar to that of wild type males of the same age. By 205 hours the pigment distribution below the postretinal pigment cells and among distorted pigment cells of other regions is irregular and resembles the condition found in the adults (Figure 6). During the entire pupal development of *lz* the pigment distribution is normal except in the structurally distorted regions where the pigment cells are irregular in shape and size. The color of the granules in abnormal areas is normal and abnormalities in eye color of such regions are the result of variations in cell size and the number of granules present. Pupation in *lz* occurs slightly later than in wild type so that in both normal and *lz* eyes pigmentation begins about 40 hours after pupation.

A complete series of pupal stages was not obtained for lz^{BS}, but examination of those available indicates that pigmentation is similar to that of lozenge. Abnormalities in the distribution and shape of pigment cells are more common with resulting irregularities in the location of pigment granules. By 190 hours a heavy concentration of dark red granules is present in the marginal ommatidia. Some pigment occurs with abnormal postretinal retinulae and a small amount of pigment has been deposited in the primary pigment cells.

The eyes of lz^{g} adults are darker in color than wild type (OLIVER 1940) and this difference becomes evident during pigmentation in the pupae. The first indication of pigment formation occurs in individuals of 170 hours; at this time a light yellow color is present in the normal cells of the basal region and in the proximal portions of the secondary pigment cells. Some scattered pigment granules occur in the postretinal pigment cells at 175 hours and pigmentation in distorted secondary pigment cells may be seen. The color in the basal region is dark brown at 180 hours. The granules in the secondary pigment cells are very dense at the basal portions and less concentrated in the distal regions. A few brown granules are present in normal primary pigment cells and scattered patches of brown pigment occur with abnormal cells in the postretina. By 195 hours the eye color is reddish-brown with the postretina and basal pigment a deep red color (Figure 7). The color becomes more intense just prior to the time of emergence; the cells just below the cornea are red-orange while the basal region is a very deep red (Figure 8).

Pigmentation in lz^{34} pupae is similar to that of glossy in final distribution of pigment and intensity of eye color. However, pigment was observed at an earlier stage in this mutant than in lz^{g}. The severe distortion and abnormality of the ommatidia in lz^{34} make the distinction of pigmentation in normal and abnormal cells difficult since only a few scattered ommatidia are normal. At 165 hours, the pigment, which is yellowish-brown in color, is limited to the region of the basement membrane. In those regions where the basal membrane is disrupted by abnormal retinulae, pigment is absent so that the basal pigment layer is not normal. During the next five hours the color becomes a deeper brown and a heavy concentration is present in the basal portion of the marginal ommatidia (Figure 9).

By 175 hours the pigment granules of the secondary pigment cells extend peripherally about one half the length of these cells. As the extension of pigment in these cells continues, the effect of the structural abnormalities becomes evident. Disrupted pigment cells show a corresponding irregularity in the distribution of the pigment. At 180 hours the pigment extends about two thirds the length of the secondary pigment cells and scattered masses of pigment have appeared in the postretinal layer of pigment cells. At this time no pigment is present around the postretinal retinulae although in individuals of 185 hours some streaks of brown pigment were observed surrounding these cells. By 190 hours the eye color is dark brown with irregularly distributed regions of reddish-brown. In those regions where pseudocones are forming some scattered pigment



FIGURES 9–12.—Unstained sections of lozenge eyes during pupal development. Carnoy's fixative, initial magnification × 200. FIGURE 9.—lz^{ja}, 170 hours. FIGURE 10.—lz^{ja}, 190 hours. FIGURE 11.—lz^{ja}, 210 hours. FIGURE 12.—lz^{3}, 190 hours.

granules can be observed, but where the cornea is flattened and the pigment cells form a layer beneath the cornea no pigment is present at this time (Figure 10). The pigment in the normal primary pigment cells is yellowish-red at 200 hours, deepening to an orange-red by 210 hours, the condition present in the adult eyes. The primary pigment cells which occur in a layer beneath the cornea vary in their pigmentation from a deep red to a yellowish-red. Although the pigment in other regions is irregularly distributed as a result of structural abnormalities the color is dark red in the secondary pigment cells and in those pigment cells of the basement membrane region (Figure 11).

The development of pigment in those mutants in which there are no normal ommatidia is closely associated with the structural abnormalities that arise during pupal development. Of the five mutants in this group, only lz^{ja} has a red eye color in the adult; the remaining mutants, lz^{3}, lz^{8}, lz^{8B}, and lz^{36}, have yellowish-brown eyes with flecks of red. The first indication of pigmentation in the eyes of these mutants occurs later than in those previously described. Individuals of 170 hours show no pigment in any regions of the eye while at 175 hours yellowish-brown granules may be seen in the basal regions of the secondary pigment cells of lz^{ja} and lz^{3}. The retinulae, which form an irregular layer beneath the pigment cells, have no pigment around them at any time during

pupal development. Each of the alleles develops a heavily pigmented rim, appearing at 180 to 185 hours and darkening with age. In lz^3 the secondary cells have brown pigment granules throughout their length by 185 hours, with the color appearing irregularly as an orange-brown after 190 hours (Figure 12). The rim is dark brown at 190 hours and becomes very dense by the time of emergence. The layer of primary pigment cells beneath the cornea is unpigmented until about 195 hours when it appears as a light brown layer, with occasional flecks of reddish brown pigment scattered irregularly.

In lz^{ts1} the basal region of the secondary pigment cells is brown at 180 hours, and after ten hours the brown granules form more distally until they are distributed throughout the length of the cells. At 195 hours the color is reddish-brown in some regions and rust colored in others; some pigment granules may be observed in the primary pigment cells at this time also. The marginal region is dark brown at 180 hours, darkening during the next ten hours and becoming reddish brown by 205 hours. The pigmentation at 205 hours is irregular through the central portion of the eye, varying from yellowish-brown to a dark reddish-brown color.

The pigmentation is lz^s, lz^{sB} and lz^{16} is identical as far as can be distinguished by the study of unstained sections. The secondary cells are irregularly pigmented in the basal regions at 185 hours with extension through the distal portion of these cells during the next ten hours. The cells are never regularly pigmented, and the appearance is abnormal, with scattering of brown granules throughout the secondary pigment cells. The primary pigment cells remain unpigmented until about 205 hours, when scattered brown granules appear. Pigmentation in the rim has begun by 185 hours, appearing as a brown color in the basal portion. By 190 hours the color is darker brown, but the pigmentation does not extend to the eye surface at this time. By 210 hours the rim is reddish brown, but the central regions of the eye retain in their yellowish-brown color.

DISCUSSION

In the development of the compound eye of *Drosophila melanogaster*, the pigment starts forming in the region of the basal pigment cells and proximal portions of the secondary pigment cells and progresses centrifugally. Elongation of the secondary cells occurs simultaneously with the outward progression of the pigment. A short time after the first appearance of pigment in the basal region, pigment granules appear in the postretinal cells and finally pigment appears in the primary cells during the period when the pseudocones are forming.

The onset of pigmentation in the different cells of the normal eye and the lozenge mutants is shown in Figure 13. By comparing the time of pigmentation it can be seen that the deposition of the pigment occurs in the same way in wild type and two of the lozenge alleles, lz and lz^g, appearing first in the secondary (S) and basal (B) pigment cells, about five hours later in the postretinal (PR) pigment cells, and finally in the primary (P) pigment cells.

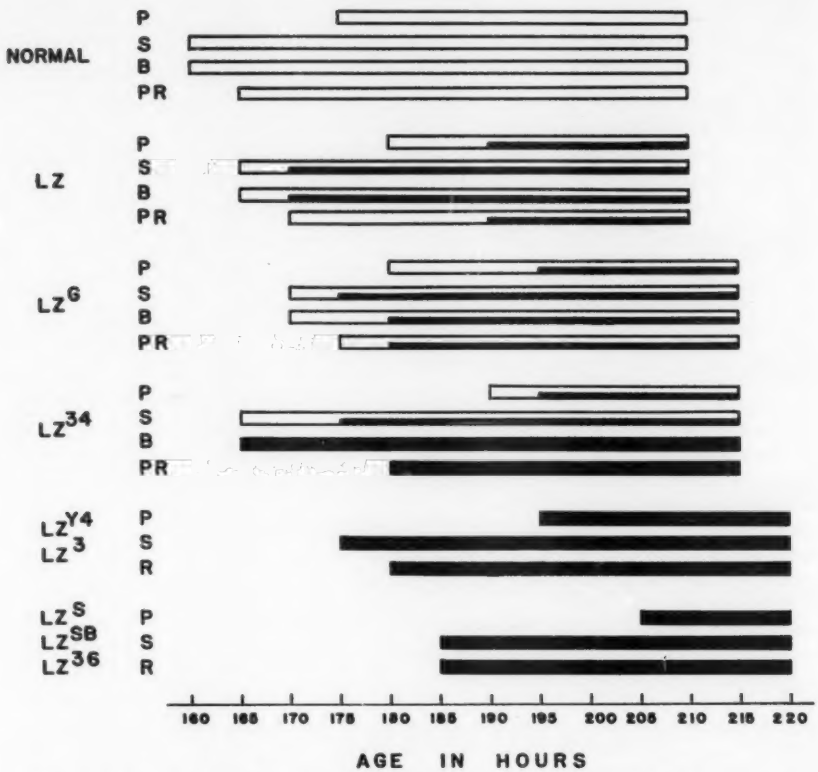


FIGURE 13.—Onset of pigmentation in normal and abnormal ommatidia in the normal eye and eight lozenge pseudoalleles. Pigmentation in normal ommatidia is indicated by the open bars and pigment deposition in abnormal cells by the solid bars. The onset of pigmentation in normal and abnormal pigment cells is indicated in hours from egg deposition. The four regions of pigment distribution are indicated as: B–basal pigment cells; P–primary pigment cells; PR–postretinal pigment; R–pigment in margin or rim; S–secondary pigment cells.

The difference in the time of appearance of the pigment in these different cells may be correlated with the morphological development of the ommatidial elements (CLAYTON 1954). Pupation is delayed in both *lz* and *lz^g*; however, since differentiation of the ommatidial cells occurs at about the normal rate, the first appearance of pigment occurs at a later time but at the same relative stage in cellular differentiation. If, however, pigmentation in normal ommatidia of the mutants is compared with pigment deposition in abnormal cells, it is seen that the pigment deposits in the abnormal cells appear from five to 20 hours later than pigment in normal cells of the same region. In *lz^34*, an allele which has only a few normal ommatidia, the distinction between pigmentation in normal

and abnormal ommatidia was observed only in the primary and secondary pigment cells. The basal and postretinal regions are so severely disrupted by structural abnormalities that no normal pigmentation was observed in these regions.

Although normally pigment appears to be heavier in the marginal cells than in other ommatidia during early pigmentation, a distinct marginal rim develops and is retained in five of the lozenge alleles. The heavy deposition of pigment in the marginal cells occurs during the developmental period when scattered pigment granules are visible in the secondary pigment cells. Although the eye colors in these five mutants, lz^{v4}, lz^{s}, lz^{s}, lz^{sB}, and lz^{s6}, differ in the intensity of the red pigment, the distribution of the pigment granules is similar. No pigment was observed during pupal differentiation in the postretinal region of the eyes and although the extreme distortion of the ommatidial cells made recognition of the basal cells difficult, no pigment was definitely located in these cells.

The more striking differences in eye color appear among those alleles with severe structural irregularities of the ommatidia. In the investigation of pigmentation in the eyes of adults of the lozenge series (CLAYTON 1957, 1958) it was found that alleles similar in the gross structural changes in their ommatidia could vary considerably in eye color. For example, lz^{v4} and lz^{s} are indistinguishable on the basis of structural abnormalities but the eyes of lz^{v4} are very dark red whereas those of lz^{s} are light brown. The distribution and number of pigment granules are similar although the amounts of red and brown pigment differ considerably in the two alleles. The development of these two mutants shows that pigmentation progresses in the same manner. The differences in eye color seem to be the result of the control of the gene over the amount of red pigment deposited in the granules during late pupal development. In lz^{s}, lz^{sB} and lz^{s6} pigmentation commences later than in the less severely affected alleles and the pigment granules remain brown until just before emergence when traces of red pigment are deposited.

The effect of the lozenge gene on the development of the compound eye seems to involve two phases, differentiation of the elements of the ommatidia and the deposition of red and brown pigments in the cells of the visual units. Differentiation occurs normally until the period of eye development in which the retinular components begin to elongate and differentiate into the various cells of the ommatidium. Structural abnormalities appear first and about ten to fifteen hours later, as pigmentation begins, variations in pigment deposition occur. In those alleles which retain some normal ommatidia, pigmentation occurs normally except for differences in the amount of red and brown pigments deposited in cells which otherwise appear to be normal. In *lz*, one of the less severe mutants, the irregularities in eye color of the adult may be correlated with the occurrence of ommatidia which did not differentiate normally and with variations in the amount of pigment produced in the abnormal pigment cells. In the lz^{g} eye, which is darker than the wild type, the amount of pigment deposited is greater

in both proximal and distal regions of the ommatidia. Other differences in the appearance of the eye are the result of structural abnormalities.

In an histological analysis of eye development in *Drosophila pseudoobscura*, COCHRANE (1936) reported that changes in eye color result from an increase in the number of granules and the intensity of the color in the granules. She described two different kinds of granules in the developing pigment cells of the wild type eyes. NOLTE (1950), however, analyzed the eye-pigmentary system in *Drosophila melanogaster* and found no indication of specificity of granules for a particular eye pigment; rather, he found evidence that the granules are carriers of both pigments simultaneously. This indicates that mutants affecting eye color influence the number of granules present, their size, or the amount of pigment present in the granules rather than controlling the production of granules specific for either red or brown pigment. EPHRUSSI (1945) studied deposition of red pigment in scarlet pupae by spectrophotometric methods. The shapes of the absorption curves change with the age of the pupae in a uniform and gradual manner that suggests that the eye colors characteristic of the different stages of development are due to changes in the composition of the pigment. The examination of both normal and lozenge eyes in pupae of different ages does not reveal a specificity of granules for either red or brown pigment, and variations in adult eye color seem to be the result of differences in the relative amounts of the two pigments deposited in the granules, the number of granules present, and the irregularities in the arrangement of the pigment cells in the mutants. An additional factor which may account for absence of pigment in some ommatidial regions is the degeneration which occurs among pigment cells in the severe alleles.

The effects of the lozenge gene on the structure and pigmentation of the compound eye have now been studied histologically in adult males, in compounds of different lozenge alleles, and in developmental stages from pupation until emergence of the adults. A direct correlation between abnormalities in the structure of the ommatidial cells and irregularities in the shape and arrangement of eye facets has been found, and the structural abnormalities of the adult eyes have been traced to irregular differentiation during pupal development of the cells which form the ommatidia. The abnormal shape and arrangement of the pigment cells are related to the distribution of pigment in adult eyes and may partially explain the amount of pigment present. Degeneration of cells in the late pupal stages among the severe alleles may result in the absence of pigment in such regions, and the irregularities in the size of the cells may account for variations in pigmentation.

Many cases of multiple phenotypic effects of a single gene have been examined in Drosophila and in other organisms. It has been demonstrated that pleiotropism may result from a single gene mutation that produces its primary effect early in development. However, not all pleiotropism has been satisfactorily related to a single developmental process. GRUNEBERG (1947), on the basis of the results obtained in developmental studies on achondroplasia in the rat, con-

cluded that pleiotropism is spurious and that gene effects may be traced to a single developmental change. SCHULTZ (1935) described the effects of a number of eye color mutants during differentiation of the compound eye in Drosophila. He considered the eye color as the result of two relatively independent aspects, the onset of pigmentation, and the increase of pigment, and observed that the fact that a given gene affects two apparently unrelated processes may indicate an unsuspected relationship or the gene may be capable of producing more than one primary effect. NOLTE (1952) described the pleiotropic effect of various alleles of the white locus and suggested that alleles act on the same or a similar substrate in different regions, which may be utilized in different ways during development. If the main product of the gene action is the type of protein, it may be utilized in the eye as a protein carrier or for pigment granules and as structural protein in the spermathecae.

The series of histological studies on the lozenge pseudoalleles have not revealed any single factor which will satisfactorily explain the multiple effects of the gene. Some secondary effects have been traced to improper cell differentiation during pupal development. ANDERSON (1945) found that reduced fertility in lozenge females may be traced to abnormalities in the development of the female reproductive structures; the sperm stored in the ventral receptacle of the female lose their motility or viability by 72 hours after insemination (OLIVER and ANDERSON 1945). The author has reported abnormalities in the differentiation of the cells composing the ommatidia of the lozenge eyes as the factor resulting in the abnormal facet appearance and, to some extent, irregularities in pigmentation. This series of studies, as well as many other investigations have not successfully found any one factor that could produce the complex phenotype of the lozenge gene. Studies by GREEN (1948), CHOVNICK and FOX (1953) and CLAYTON (1954a) have failed to show a correlation between the actions of the lozenge pseudoalleles and the loci to which they have been assigned by GREEN and GREEN (1949). Although some of the morphological changes may be traced to a single causal factor, variations in the amount of pigment deposited in eyes that are similar morphologically and differences in the interactions of the alleles when in compounds seem to indicate that the gene has more than one primary effect or that the basic relationship between the various phenotypic expressions of the gene has not yet been found.

SUMMARY

Pigmentation during the development of pupae is described for *Drosophila melanogaster* and eight of the lozenge pseudoalleles. The onset of pigmentation, deposition of granules in normal and abnormal pigment cells, and variations in pigment deposition resulting from structural abnormalities are given.

In *lz* and lz^g, pigmentation in normal ommatidia progresses in the same way as in the wild type eye but irregularities in deposition of pigment occur wherever ommatidia are abnormal in shape or arrangement. Extreme distortion of ommatidial cells in lz^{y4}, lz^3, lz^s, lz^{sB}, and lz^{36} pupae resulted in scattered pigment de-

posits in secondary pigment cells with most of the red and brown pigment deposited in a layer beneath the cornea. The distribution of pigment granules is correlated with the distortion of pigment cells, but the amount of pigment present in the cells may vary in alleles which histologically are similar. Abnormal cell differentiation is the primary cause of some of the irregularities in eye color, but the amount of pigment produced during pupal differentiation is either the result of some other independent effect of the lozenge gene or the relationship between these two phases of development has not yet been found.

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RADIATION-INDUCED AND GENETICALLY CONTRIVED DOMINANT LETHALITY IN HABROBRACON AND DROSOPHILA

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THE condition of dominant lethality is fulfilled when a haploid nucleus has been altered in such a manner that upon combination with a normal haploid nucleus the resulting heterozygote dies either immediately or eventually. Of the various mutation classes induced by physical or chemical mutagens, dominant lethality occurs with the highest frequency and is the simplest to measure.

Three different types of dominant lethality are recognizable in Habrobracon by phenotype (ATWOOD, VON BORSTEL, and A. R. WHITING 1956). Type I dominant lethality, which occurs in oocytes irradiated in the first meiotic metaphase or prophase, is the most predominant and is characterized by a depression of mitotic rate with complete cessation of mitosis after the second or third nuclear division. This is followed by a curious syndrome involving production of Feulgen-negative nuclei that eventually enlarge (VON BORSTEL 1955). In the present investigations we found that most of the dominant lethality induced in sperm is also of this type. Type II dominant lethality, which is inducible in metaphase I oocytes, is characterized by death after blastula formation and before hatching in the haploid (unfertilized) egg, but predominantly after hatching in the irradiated egg fertilized with normal sperm. Type III dominant lethality, which is inducible in prophase I oocytes, is characterized by death after blastoderm formation and before hatching whether or not the egg is fertilized with normal sperm.

Since progeny tests of dominant lethal heterozygotes are impossible, experiments of a reconstruction type were chosen as the simplest analytical procedure for interpreting the nature of radiation-induced dominant lethality. Since chromosome imbalance has been regarded as the main source of dominant lethality (MULLER 1954), the time and characteristics of death of aneuploids from triploids and translocation heterozygotes were compared with the dominant lethal embryos induced by radiation. Both Habrobracon and Drosophila were used in order that (1) the various types of dominant lethality could be compared from one species to another, and (2) the unique advantages of each genetic material could be used for study of different aspects of the problem.

MATERIALS AND METHODS

The wasp *Habrobracon juglandis* is parasitic on the larva of *Ephestia kühniella*, the Mediterranean flour moth. For hatchability and adult survival studies, the

[1] Operated by the Union Carbide Corporation for the United States Atomic Energy Commission.

wasps were transferred twice daily to two-inch stender dishes, each with a single new unstung caterpillar. The wasps were kept in incubators maintained at the optimal temperature of 30° C. The egg number was recorded, and dislodged eggs were placed back on the paralyzed host. Thirty hours later, hatchability counts were taken and the stage of death of the dead embryos was recorded. Classification of dead embryos was greatly facilitated by prevention of shriveling through the elevation of humidity with a pan of water in the incubator. After ten days, postembryonic deaths and surviving adults were counted. Habrobracon stocks No. 33 virgins and 17 o^i males were used for the radiation experiments.

The hatchability procedure used for *Drosophila melanogaster* embryos was essentially that used by BAKER and VON HALLE (1953). Oregon-R males were irradiated and pair-mated with Oregon-R virgins. After 24 hours, the males were removed. Each female was placed on charcoal-agar medium to facilitate egg counting and, 24 hours later, was transferred to a fresh vial. The dead embryos were fixed and stained for cytological analysis with a whole-mount Feulgen procedure in which the embryos are aligned on cover slips (VON BORSTEL and LINDSLEY 1959). The procedure was used for the translocation heterozygote and triploid studies as well. Care was always taken that dead embryos studied cytologically were from vials in which the hatchability was at least the lowest expected value, to avoid, insofar as possible, selection of unfertilized eggs.

Triploid females

Habrobracon: In Habrobracon, hemizygous individuals or individuals homozygous for the same sex alleles become males; heterozygotes become females (P. W. WHITING 1943). Half the fertilized eggs from inbred stocks would normally become diploid males except that most of these individuals die just before or just after hatching. A few do become adults but are usually infertile. Occasional (about one in ten) diploid males are fertile and have diploid sperm. Daughters from the cross of a diploid male with a normal female are always triploids. The offspring from a triploid virgin female would have anywhere from the haploid number of chromosomes (10) to the diploid number (20). Aneuploid embryos from five different triploids were examined.

Drosophila: Triploid Drosophila were first described by BRIDGES (1921). Their origin is usually unknown. One probable common source is a tetraploid piece of gonial tissue that occurs in the mother and gives rise to diploid ova. Aneuploid embryos from two different spontaneous triploids crossed with Oregon-R males were examined.

Translocations

Habrobracon: Since translocation stocks were unavailable in Habrobracon, a method was devised for their detection in genetically unmarked stocks. The translocations were induced with X-rays in males and determined by testing for the resultant inherited partial sterility. Several males from each F_1 female showing reduced egg viability were mated to wild type females, and the F_2 females were tested for reduced hatchability. Those females showing reduced hatch-

ability are considered as being heterozygous for a translocation. (A detailed presentation of this material will be published elsewhere.) Aneuploid females from four different stocks showing inherited partial sterility were examined.

Drosophila: Two translocations were used in this study, T(2;3)S^M and T(2;3)*Xa*. In each case stocks bearing these translocations were crossed to Oregon-R; the daughters bearing the translocations were backcrossed to Oregon-R males, and the dead embryos collected and studied. The specific stocks used were T(2;3)S^M *Cy c3G Sb Ubx/st c3G ca* and *In(3LR) Ubx*^{130}, *Ubx*^{130} e^s/T(2;3)*Xa*.

Irradiation procedure

A G.E. Maxitron-250 unit was used to administer the X-radiation. The machine, operated at 250 kvp, 30 ma, had a tungsten target, 0.1 mm of Al inherent and 3 mm of Al added filtration (half-value layer, 0.40 mm of Cu). Dose rates of 330, 675, 1000, and 1885r/minute were used at 39, 31, 23, and 16.5 cm, respectively, in different experiments. Habrobracon eggs in the first meiotic metaphase were irradiated at the lowest rate, 330r/minute; Habrobracon sperm, 675r/minute; Drosophila sperm, 1000r/minute; and Habrobracon eggs in the first meiotic prophase, 1885r/minute.

METHODS OF ESTIMATING LETHALS

Equations for estimating the frequency of dominant and recessive lethal mutations in Habrobracon were derived by ATWOOD *et al.* (1956). Since these equations are more generally useful than at first indicated, and since they are used throughout this paper, some of them are listed here and their properties discussed.

The frequency of embryo dominant lethal mutations, D, that depress hatchability when sperm are irradiated can be estimated by

$$D = 1 - \frac{m - V_u\,(1 - f)}{f} \tag{1}$$

where m is the hatchability frequency of eggs from mated females, V_u is the surviving proportion of unfertilized eggs (estimated from unmated controls), and f is the proportion fertilized. Adult survival data can be inserted in Equation (1) to estimate D', the frequency of total dominant lethal mutations; m' and V'_u for adults would correspond to m and V_u for embryos.

In irradiated eggs, the frequencies of fertilized eggs dying at any stage of development, s_f, were computed from the data in Table 1 by

$$s_f = \frac{s_m - s_u(1-f)}{f} \tag{2}$$

where s_m is the proportion of embryos from mated females that die at a particular stage of development and s_u is the proportion of embryos from unmated females that die at the same developmental stage. Equation (2) is of the same form as Equation (1). When the equation is used for all stages of embryonic, larval, and pupal development, it is a step-by-step analysis in which the effect of fertilization

TABLE 1

Survival and stages of death of embryos after irradiation of Habrobracon gametes

Stage irradiated and dose (r)	Viable embryos/total	Number dying at each stage of development*													Adults	
		Embryos					Larvae				Pupae					
		1	2	3	4	5	6	7	8	9	10	11	12	13	Male	Female
0 (875 control)	1616/1631	1	1	7	1	5	1	70	9	6	9	10	63	0	1448	0
Sperm																
2,000	307/549	165	2	37	20	18	9	6	5	4	2	3	6	3	157	112
4,000	214/534	247	1	32	31	9	28	11	1	1	8	7	0	1	136	21
4,000	190/820	467	5	14	103	41	25	10	8	4	2	5	6	2	69	59
6,000	130/595	417	4	16	21	7	7	3	3	1	0	0	8	2	98	8
Metaphase I eggs																
875																
Unmated	135/476	190	13	118	15	5	5	6	1	1	6	4	7	0	105	0
Mated	244/570	209	10	68	18	21	20	15	3	3	6	10	13	0	55	119
1,100																
Unmated	111/750	425	22	121	56	15	9	5	3	3	9	3	4	0	75	0
Mated	122/576	314	21	25	57	37	17	1	3	5	8	3	7	2	25	51
Prophase I eggs																
15,000																
Unmated	211/537	236	29	25	21	15	33	7	4	4	2	0	10	1	150	0
Mated	232/531	227	6	12	18	36	22	5	5	6	3	3	14	1	53	120
18,000																
Unmated	151/506	221	24	83	16	11	17	12	8	3	3	1	5	0	102	0
Mated	229/607	250	15	39	50	24	13	21	5	2	9	1	3	0	54	121

* The numbers refer to stages of development; for further description, see text.

on the expression of death of irradiated eggs can be compared with that of unfertilized irradiated eggs. In all graphs describing the radiation effects, the frequency of the stages of death of fertilized eggs was computed from the data in Table 1; the negative values obtained in a few instances are caused by random fluctuations in the frequency of death at the various stages of development.

In previous publications (ATWOOD *et al.* 1956; CLARK and MITCHELL 1952; A. R. WHITING and MURPHY 1956), the value of f has been taken from controls because adults in experiments were usually not counted and f has been assumed usually to be from 0.60 to 0.67. For an absolute determination from the experiment itself, the proportion fertilized, f, is given by

$$f = \frac{q + V'_u - m'}{V'_u} \tag{3}$$

where q is the ratio of surviving adult females to total eggs (ATWOOD *et al.* 1956). Equation (3) can be restated simply as

$$f = 1 - \frac{p}{V'_u} \tag{4}$$

where p is the ratio of surviving adult males from mated females to total eggs from mated females. This formulation is valid under any condition of lethality, and is used in the data analyses throughout except for the triploid females, where a value of f of 0.67 was used because no adults survived. Equation (3) also was used to estimate f values for the irradiated sperm given in Table 1 for computing Figures 2 and 5. Except where data from unmated control females indicated a considerable discrepancy, V_u was taken to be 0.95 and V'_u was taken to be 0.90; variations from these values as much as ten percent will not have more than a four percent effect on f or D.

CLASSIFICATION

Habrobracon embryos: The stage of death can be determined with reasonable accuracy on the unfixed unstained Habrobracon embryo. The egg chorion is two microns thick and is transparent. The embryos are classified in five different categories, Stages 1, 2, 3, 4, and 5 (Figure 1). Stages 3 and 4 can be further subdivided, although the stages are not separated in the data reported here. The stages of death are sequential in embryogenesis, but the absolute times of death during embryogenesis have not been accurately determined.

Stage 1 is the embryos in which death occurs during karyokinesis before the blastoderm forms; the embryos have a mottled white appearance and one end, or both, is filled with a clear fluid. Stage 2 is the blastula stage up to the beginnings of tissue differentiation and is characterized by two homogeneous white bands. Embryos in Stage 3 are those in which the yolk mass is changing from white to yellow and the yolk is beginning to become more centrally located. Stage 4 is a nearly fully developed embryo and can be readily distinguished by the presence

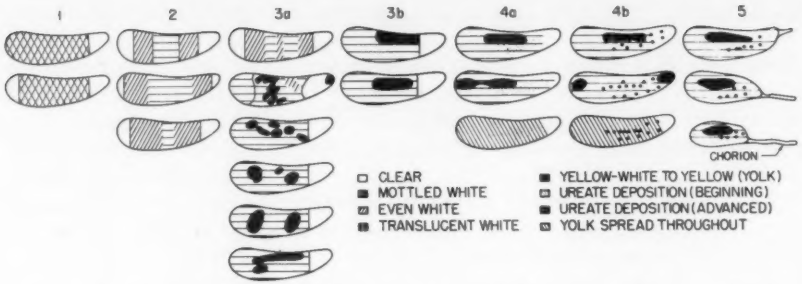

FIGURE 1.—Stages at which radiation death is expressed during the development of Habrobracon embryos.

of tracheal elements and ureate cells. Stage 5 is made up of fully developed embryos that die at any time during the hatching process. Stage 3a is distinguished from 3b by the heterogeneous distribution of the yolk in the former. Stage 4a embryos are those in which tracheal elements are just beginning to form and 4b embryos have fully formed tracheal elements and ureate cells. The gut occasionally ruptures in Stage 4 embryos, and the yolk in these may be homogeneously distributed throughout or be somewhat concentrated in the posterior end of the embryo. The embryos in Figures 3, 4, and 5 were classified in this manner.

At random intervals, the embryos were fixed and stained. For the most part, this was done for microscopic examination of embryos that had died in Stage 1 (preblastula). Five different stages of death in fixed and stained Habrobracon embryos have been described (A. R. WHITING, CASPARI, KOUKIDES, and KAO, 1958) that correspond roughly to the stages determined on the unstained embryos. Table 2 shows the same batch of irradiated dead embryos staged unstained, then fixed, stained, and staged by cytological methods. No attempt was made to

TABLE 2

Comparison of methods for determining time of death of Habrobracon embryos irradiated as Metaphase I eggs with 1100r

	Eggs from virgins				Eggs from mated females			
	Unstained		Stained		Unstained		Stained	
Stage of embryonic development	Number analyzed	Percent of total	Number analyzed	Percent of total	Number analyzed	Percent of total	Number analyzed	Percent of total
1	362	69.4	136	73.5	377	65.9	113	67.2
2	15	2.9	8	4.3	28	4.9	9	5.4
3	90	17.2	30	16.2	56	9.8	18	10.7
4	45	8.6	8	4.3	69	12.1	15	8.9
5	10	1.9	3	1.6	42	7.3	13	7.7
Total	522	100.0	185	99.9	572	100.0	168	99.9

keep track of the individual embryos from the unstained through the stained states, and the two analyses were done by different investigators. The good agreement demonstrates that the much faster analysis of unfixed, unstained material is reliable. In every other case in which microscopic analysis was performed, the embryos were first classified with respect to stage of death and then arranged on cover slips in a manner that allowed direct individual comparison with the stage of death as determined on the unfixed, unstained embryos. Microscopic analysis at intervals provided a control for the much faster analysis on the unfixed, unstained embryos.

Habrobracon larvae and pupae: Although Habrobracon larvae go through five instars, it is convenient to assign only four stages (6, 7, 8, and 9) to the larval developmental period. Stage 6 is composed of larvae that die after hatching but that never feed. Stage 7 larvae feed but die at the end of the first larval instar or at the beginning of the second. Stage 8 larvae die in the intermediate stages of growth. Stage 9 larvae have finished feeding and die before spinning a cocoon.

Stage 10 is composed of larvae that have spun a cocoon, and pupae in stages too early for the sexes to be discriminated. Stage 11 pupae are white or just beginning to darken; in these, sex can be determined. Stage 12 pupae are well developed in body coloration but the wings have not expanded; the antennae may or may not be still attached to the thorax. Stage 13 pupae have expanded wings and appear ready to eclose.

Drosophila embryos: Analysis of dead Drosophila embryos was done on fixed and stained whole-mount preparations only. The embryos were classified in three categories: Stage 1 is made up of embryos that died during karyokinesis, before enough nuclei were present to form a blastoderm. Stage 2 consists of embryos that died at or just before blastulation. Stage 3 is comprised of embryos that died after tissue formation began but before hatching. Even though the last category is a broad one, our interest is particularly centered on Stage 1 since it is most predominant after irradiation of the gametes.

RESULTS

Radiation-induced dominant lethality

Type I lethality, that expressed before blastoderm formation, is predominant when Habrobracon or Drosophila gametes are irradiated with doses that are lethal to the majority of embryos. This is shown in Figure 2, where dose-action curves compare the proportion of embryos dying before blastoderm formation with the embryo hatchability. With Habrobracon sperm (2c) or metaphase I eggs (2b) or Drosophila sperm (2d), the dose-hatchability responses are approximately exponential; to a large extent, this is a manifestation of the simple exponential nature of the Type I lethality in response to dose. The Habrobracon eggs irradiated in prophase I exhibit two-hit kinetics (Figure 2a). Drosophila eggs irradiated in prophase I also show that two hits are requisite for lethality (PARKER 1955; KING, DARROW, and KAYE 1956; KING 1957). The Habrobracon

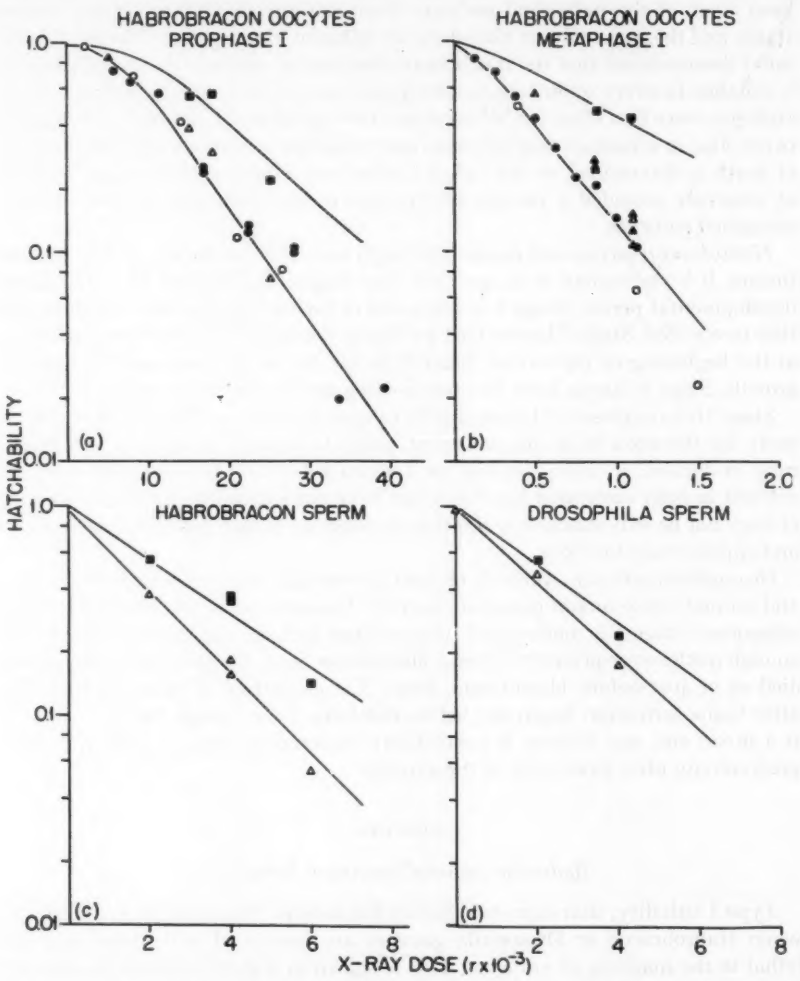

FIGURE 2.—Dose-action curves for Habrobracon prophase I (a) and metaphase I (b) oocytes and Habrobracon (c) and Drosophila (d) sperm. Hatchability data are from A. R. WHITING, 1945 b ●; KENWORTHY, 1956 ○; ATWOOD *et al.*, 1956 ▲; and these experiments Δ. ■ 1.0 — proportion dying before blastoderm formation.

oocyte data are from unfertilized eggs; induced recessive lethals contribute about 0.10 of the deaths to both metaphase I eggs and prophase I eggs at the 0.37 survival level.

Cytologically, the Type I lethality in Habrobracon is characterized by meiosis at the normal rate and then by an abrupt depression in the rate of mitosis, with complete cessation at the second or third division. A few hours later, anomalously Feulgen-negative nuclei arise, which eventually enlarge (VON BORSTEL 1955). In Drosophila, the Type I syndrome is similar in that no or few Feulgen-positive nuclei are seen; however, Feulgen-negative nuclei seem not to be produced after the cessation of mitosis. Absolute criteria for distinguishing dominant lethal embryos from unfertilized eggs have not been established in Drosophila.

That Type I lethality is truly dominant is demonstrated in Figures 3 and 4, where it can be seen that the time of expression of the early death induced in a Habrobracon egg nucleus is affected only slightly by fertilization with a normal sperm. The early expression of death is represented by the first peak in the graph.

Type II dominant lethality, which is induced at only the first meiotic metaphase and whose expression is delayed by fertilization, is illustrated in Figure 3. There is a consistent but slight depression in the frequency of Stage 1 deaths after fertilization but the most marked effect is on the Stage 3 lethals. This group is depressed dramatically, and the expression of death then occurs as a long slow wave that reaches a maximum during late embryogenesis and the early larval period. The frequency of death then declines but rises again slightly during the midpupal period. At the higher dose level of 1100r (Figure 3), where there is an average of about two inactivating events per nucleus, Stage 3 in the unfertilized group is depressed by absorption into the large number of Stage 1 deaths; and in the fertilized class, most of the expression of delayed death occurs at the earlier stages of development. Delay is therefore inversely correlated with dose. It can be seen, however, that a conditional delay is still expressed during the early larval period (Stage 6 in Figure 3b).

An obvious shift of time of death during embryogenesis after fertilization occurs with both the Type II dominant lethality and the Type III dominant lethality, which is lethal after blastulation and before hatching whether or not the eggs are fertilized (VON BORSTEL, ATWOOD, and A. R. WHITING 1955). The different terminology is retained, however, since (1) the delay does not extend into the posthatching stages in the eggs fertilized subsequent to irradiation at prophase I, where Type III lethals are typically induced, (2) the stages of meiosis that were irradiated to induce Type II and Type III lethality are vastly different in both morphology and radiation sensitivity, (3) the patterns of frequency of postblastulation death differs among eggs irradiated in prophase I and metaphase I, (4) it is conceivable that the basis of Type II dominant lethality may be different from Type III lethality, and (5) though there still remains a very real possibility that Type II and Type III are a continuum (see DISCUSSION), the less marked expression of delay in prophase I irradiated eggs has the heuristic value of indicating that a more severe damage must take place in prophase.

Type III dominant lethality is shown in the data from irradiation of eggs in the first meiotic prophase (Figure 4). Again, fertilization with normal sperm causes a slight depression of the Stage 1 lethals. The frequency distribution pattern of

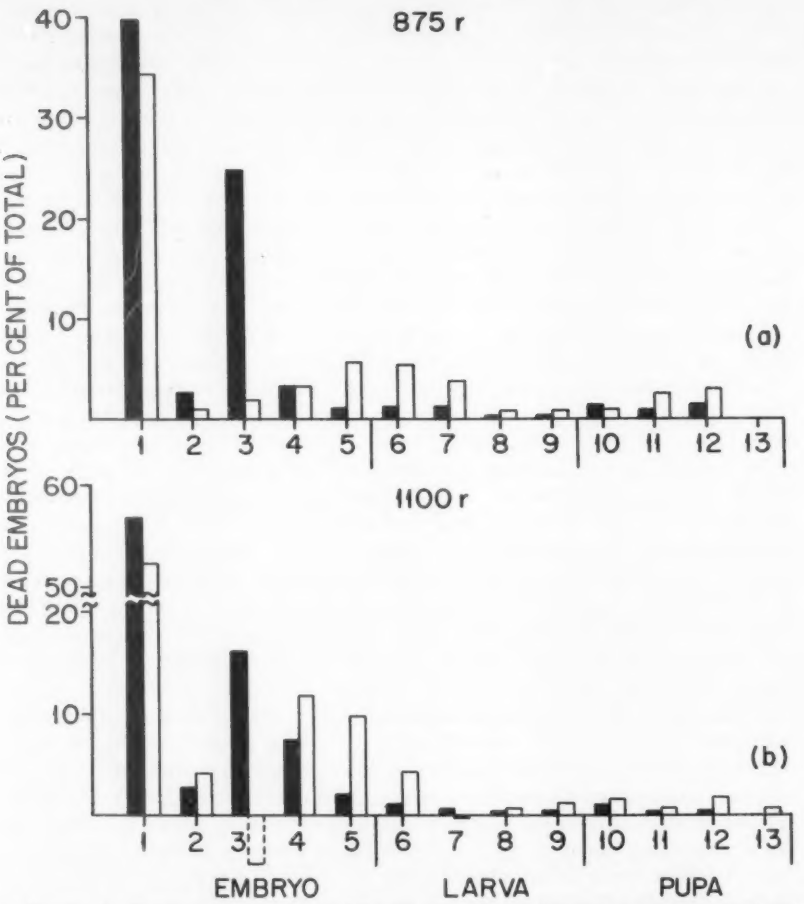

FIGURE 3.—Frequencies of death at different stages of development of embryos irradiated with 875r (a) and 1100r (b) as eggs at the first meiotic metaphase. Solid columns, unfertilized; open columns, fertilized with normal sperm.

postblastulation death (Stage 2 on), stage by stage, among the unfertilized eggs is modified from that characteristic of the deaths from irradiation of metaphase I eggs (Figure 3a, b). It is interesting that the increased frequency in death at Stage 6 not only was increased over those found in irradiated metaphase I eggs but is as high as or higher than the frequency of fertilized prophase I eggs dying at the same stage. The frequency of death at Stage 6 of the unfertilized and fertilized embryos irradiated at prophase I possibly accounts in part for the absence of Type II dominant lethality induced in irradiated prophase I eggs.

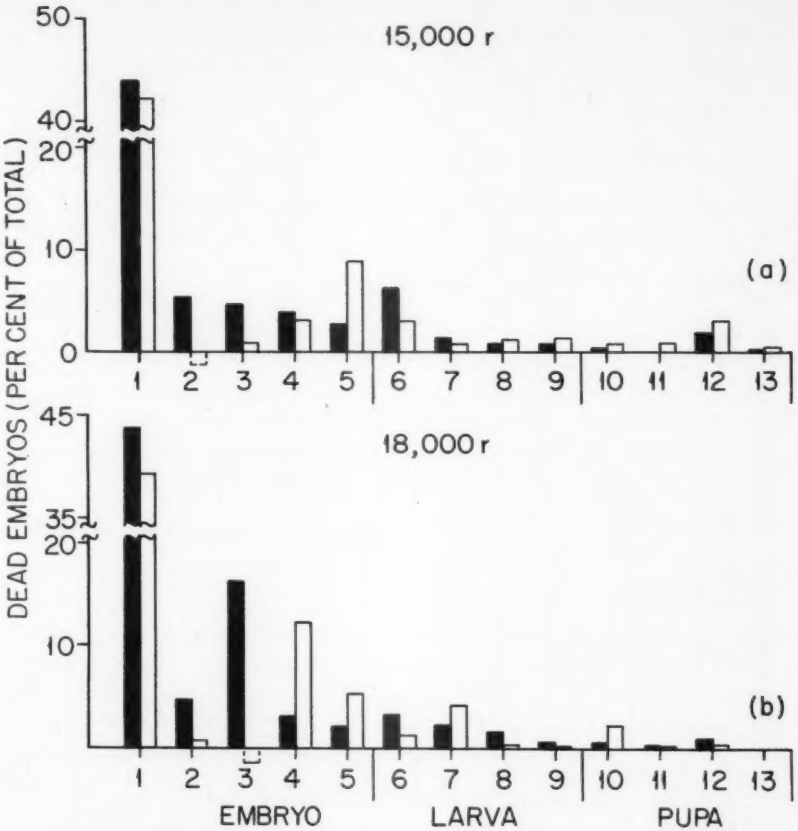

FIGURE 4.—Frequencies of death at different stages of development of embryos irradiated with 15,000r (a) and 18,000r (b) as eggs at the first meiotic prophase. Solid columns, unfertilized; open columns, fertilized with normal sperm.

When sperm are irradiated, many deaths occur during the larval stages, particularly at 4000r (Figure 5). This indicates that a certain amount of Type II dominant lethality is probably induced in sperm. Two experiments at 4000r (Figure 5b, c) are shown to illustrate the variability that can occur from experiment to experiment. At 6000r (Figure 5d), the frequency of deaths during all larval and pupal stages is depressed, presumably because the deaths at Stage 1 account for nearly 90 percent of all deaths.

Genetically contrived dominant lethality

None of the embryos from the five mated or unmated triploid Habrobracon females reported here survived past the larval stage of development (Table 3),

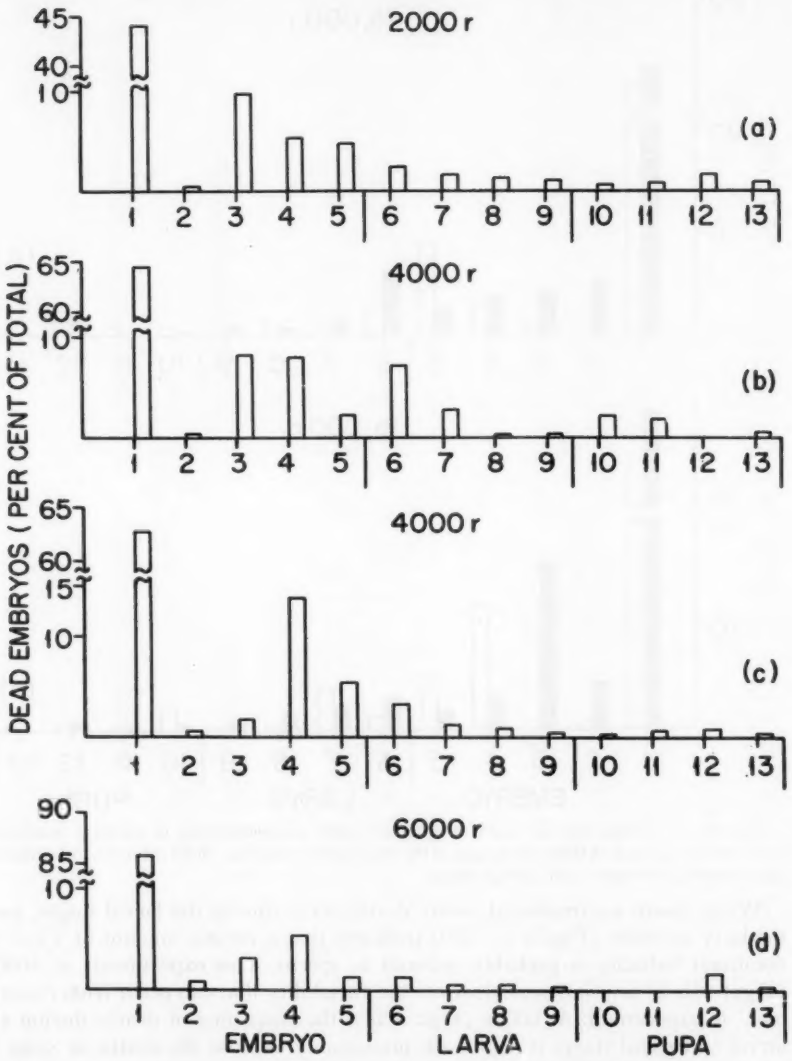

FIGURE 5.—Frequencies of death at different stages of development of embryos from normal eggs fertilized with irradiated sperm. Replicate 4000r experiments (b and c) indicate inherent variability.

TABLE 3

Stage of death of gametes from triploid Habrobracon females

		Stage of development at death								
		Embryos					Larvae			
	Viable embryos/total	1	2	3	4	5	6	7	8	9
Unmated										
1	0/17	..	..	7	10	..	..	..	..	..
2	0/50	6	1	31	12	..	..	..	..	..
Mated										
3	0/39	2	..	8	21	8	..	..	..	..
4	2/37	3	..	1	20	11	..	1	..	1
5	1/41	4	1	4	23	8	..	1	..	..

but haploid male survivors have occasionally been found (A. R. WHITING, personal communication). All embryos from the two triploid Habrobracon virgins died before hatching and, predominantly, after blastoderm formation (Figure 6). The few embryos that died before blastoderm formation (in both the fertilized and unfertilized groups) were stained and examined. All had gone through at least eight cleavages and all had Feulgen-positive nuclei and were therefore not comparable to radiation-induced Type I lethal embryos. When the females were mated with normal males (in this case, haploid brothers), the embryos died slightly later in development, but the majority died before hatching (Figure 6).

A mated Drosophila triploid female has a high likelihood of producing some viable offspring since the haploid number is four. Also segregation in a Drosophila triploid is not random, and a balanced chromosome complement is obtained less often than expected (BEADLE 1935). SANDLER and NOVITSKI (1956) called this nonrandom segregation in triploids the "crowding effect" and they argue that an assumed general homology shared by all the chromosomes provides a consistent explanation for the phenomenon. Furthermore, embryos trisomic for chromosome 4 are viable, and the X chromosome as a trisomic allows enough viability for about two thirds of the animals of this constitution to hatch and some of those hatching to survive larval and pupal life and become superfemales. Of the embryos from triploid females that did not hatch, over 90 percent died at the blastoderm stage or later (Table 4). Those dying earlier could have been unfertilized eggs.

TABLE 4

Stage of death of gametes from triploid Drosophila females

	Stage of development at death			
Viable embryos/total	Preblastoderm	Blastoderm	Postblastoderm	Hatching
59/365	13	16	124	3

Dead embryos were sampled for frequency reaching each stage.

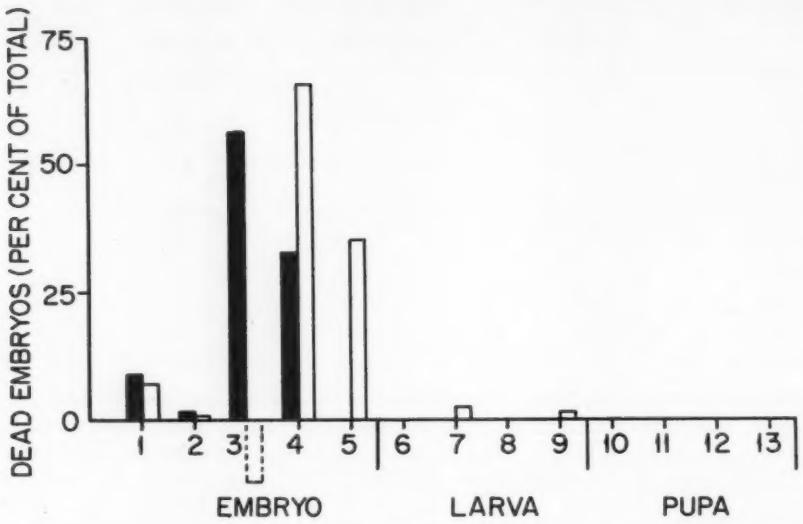

FIGURE 6.—Frequencies of death at different stages of development of aneuploid eggs from triploid Habrobracon females. Solid columns, unfertilized; open columns, fertilized with normal sperm.

During meiosis in a translocation heterozygote, the chromosome segregation pattern is such that 50 percent or more of the gametes have a deficiency for a chromosome segment and a duplication of another chromosome segment. When aneuploid eggs were oviposited by virgin Habrobracon translocation heterozygotes, the embryos died before hatching (Figure 7). Wasps from aneuploid eggs fertilized with normal sperm died predominantly after hatching, during the larval or pupal stages. These closely resembled in action the Type II dominant lethals induced by radiation in metaphase I eggs: the embryos from unfertilized eggs died with reasonable specificity during mid-embryogenesis, whereas the death pattern of all embryos from fertilized eggs tended to rise with a long, slow wave that had its highest peak just before, during, and after hatching and another small peak at mid-pupation. With all five translocations, most of the embryos died during embryogenesis after blastoderm formation; most of them had many cells (50,000 or more) with well-differentiated cell membranes. This is especially remarkable since, presumably, a large piece of chromosome is missing from the genome in each instance. These genes were therefore not necessary for development up to that time. The embryos from translocation heterozygotes that did die in Stage 1 were sampled. The few that were fixed and stained did not appear to have died in the manner characteristic of the radiation-induced Stage 1 dominant lethals; that is, more than seven nuclear divisions had occurred and the nuclei were Feulgen positive and did not swell.

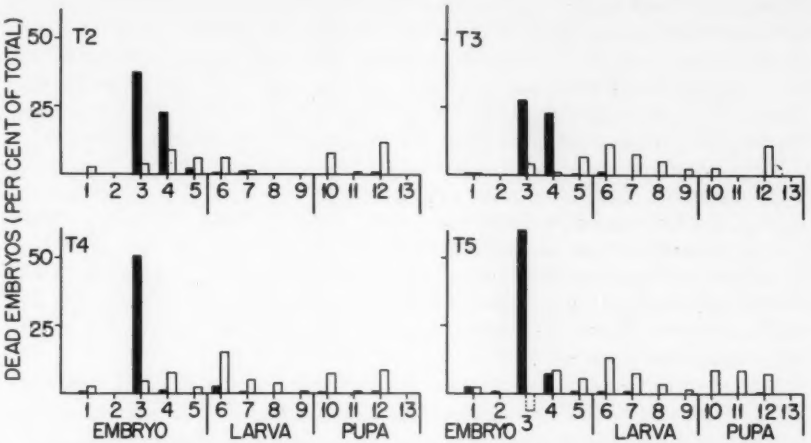

FIGURE 7.—Frequencies of death at different stages of development of aneuploid eggs from four different Habrobracon translocation heterozygotes. Solid columns, unfertilized; open columns, fertilized with normal sperm.

Of the 297 dead embryos from two different Drosophila translocation heterozygotes, 93.1 percent died at the blastula stage or later (Table 5). Those that died before blastula formation (corrected for those lost) represent only 5.4 percent of the total embryos and possibly were unfertilized eggs.

DISCUSSION

The aneuploid embryos from triploid virgin Habrobracon females provide a unique opportunity for testing the effect of extreme genetic imbalance on embryogenesis. Both the haploid and diploid embryos develop normally; embryos from a triploid virgin will contain anywhere from the haploid to the diploid number of chromosomes. If anything as drastic as radiation-induced loss of several chromosomes should bring about Type I dominant lethality, it should be mimicked precisely by aneuploids from triploid virgins. Not one of the embryos

TABLE 5

Stage of death of gametes from Drosophila female translocation heterozygotes

Translocation	Viable embryos/total	Dead embryos			
		Preblastoderm (little or no chromatin)	Blastoderm	Postblastoderm	Lost during fixation and staining procedures
Xasta	192/371	10	3	146	20
Star of Muller	285/470	10	2	126	47

resembled a Type I lethal; therefore, it follows that Type I dominant lethality is not caused by the loss of one or more chromosomes. The same argument can be advanced for the genetic imbalance provided by the segregation of translocation heterozygotes, except that the loss is less extreme. But with the translocations, the effect of a hypohaploid genome on development can be assayed as well as the hypodiploid genome, and there again the Type I lethality is not mimicked.

The aneuploid embryos from the triploids can be regarded as resembling Type III lethals since, having the hypodiploid set (between 10 and 20 chromosomes), most of them died after blastula formation and before hatching. When the eggs were fertilized, the chromosome number was between 20 and 30, and though time of death shifted to later embryonic stages, most of the embryos still died before hatching. The translocation heterozygotes produced offspring that died in a manner directly analogous to the Type II dominant lethals induced with radiation at metaphase I.

A class of radiation-induced lethality comparable to the Type II dominant lethality has not been demonstrated in Drosophila and is not demonstrable except for the sex chromosomes. Therefore, all postblastulation-prehatching deaths described here are by definition analogous to the Habrobracon Type III class. This class is seen in the dose-action curve of Drosophila sperm (Figure 2d). The postblastulation-prehatching dominant lethals comprise the area between the two curves, and, interestingly enough, occur at a lower frequency than postblastulation lethals in Habrobracon. Among possible explanations are that, with the lower chromosome number in Drosophila, chromosome aberrations are less likely if the target for Type I lethality is of a nearly constant size from Habrobracon to Drosophila, or that sperm are inactivated at low doses in Drosophila and that some of the Type I deaths are actually unfertilized eggs (see KAPLAN 1958; D. L. LINDSLEY, C. W. EDINGTON, and E. S. VON HALLE, unpublished). The difference between the curves when the Habrobracon eggs are irradiated (Figure 2a, b) is exaggerated by induction of any recessive lethals, since recessive lethal mutations cause death predominantly after blastulation (A. R. WHITING *et al.* 1958) and the metaphase I and prophase I egg data used for Figure 2a, b are from unfertilized eggs.

In addition to the genetically contrived embryo dominant lethals from triploids and translocation heterozygotes, Drosophila embryos were constructed that lacked the X and Y chromosomes with their associated nucleoli (VON BORSTEL and REKEMEYER 1958). These embryos came from the cross of attached-X females (XX/0) with attached-XY males (XY/0). Approximately 25 percent of the offspring have neither the X nor the Y chromosome but do retain the complete autosomal complement. These embryos die after the tenth to twelfth mitosis, many divisions after and under entirely different circumstances from the radiation-induced Type I dominant lethal Drosophila embryos. This is an important consideration since GAULDEN and PERRY (1958) have shown that ultraviolet microbeam irradiation of the nucleolus of the grasshopper neuroblast permanently inhibits mitosis. In Drosophila, lack of the nucleolus does not mimic

the primary radiation lesion. This does not exclude the nucleolus or nucleolus-organizer region as the radiosensitive target in Drosophila but does impose the restriction of nucleolar alteration rather than nucleolar inactivation. In eggs irradiated in the first meiotic prophase, the nucleolus is present, but only the nucleolar organizer is present during the first meiotic metaphase and in sperm. Type I dominant lethality is identical for eggs irradiated in metaphase I or prophase I or for irradiated sperm. This indicates that, should the nucleolus be involved, which we believe unlikely, the nucleolar organizer rather than the nucleolus would have to be considered as the primary target.

The classification of radiation-induced dominant lethals presented in this paper is phenotypic rather than causal. Since a relatively clear-cut separation of the phenotypes exists and since certain of the induced dominant lethal types can be mimicked, provisional explanations for the three types can be presented. These are regarded as nuclear effects since radiation damage to the cytoplasm is considered to be without influence; numerous reasons for this have been listed elsewhere (Atwood *et al.* 1956). Furthermore, separate inactivation of the Habrobracon nucleus and cytoplasm by ionizing radiation has shown that one α particle striking the nucleus can bring about death of the embryo (Rogers and von Borstel 1957) whereas 21×10^6 α particles must strike the cytoplasm of individual eggs to inactivate 90 percent of a batch of eggs (von Borstel and Rogers 1958). Since the egg cytoplasm is tremendously resistant to the radiation and since cytoplasmic damage yields a recognizable syndrome, cytoplasmic injury can be considered as contributing nothing to the three classes of dominant lethality discussed here. For Drosophila, Ulrich (1955, 1956) has shown that the egg nucleus is far more sensitive to X-radiation than the cytoplasm.

Type I dominant lethality: This results in death before the blastoderm is formed and, both in Drosophila and Habrobracon, is distinctive in appearance. In neither organism can the Type I dominant lethality be mimicked by loss of chromosomes or chromosome parts, nor, in Drosophila, by the loss of the sex chromosomes with their associated nucleoli. It has been suggested, for Habrobracon, that this kind of dominant lethal, in which mitosis is permanently inhibited at the second or third nuclear division, is associated with a defect in deoxyribonucleic acid (DNA) synthesis since the Type I syndrome is associated with the production of Feulgen-negative nuclei that eventually enlarge (von Borstel 1955). Other than inferred evidence from irradiation of other organisms that DNA synthesis is blocked by radiation or mustards while synthesis of ribonucleic acid and protein are not (Bodenstein and Kondritzer 1948; Herriott 1951; Kanazir and Errera 1954; Harold and Ziporin 1958a,b), the data to support such a hypothesis are limited. Furthermore, Kelly (1957) has compiled an impressive amount of evidence indicating that inhibition of DNA synthesis by radiation is secondary to an inhibition of mitosis. Cave and Brown (1957) suggest that the most likely explanation for the dominant lethality that occurs very early in Lilium is genetic imbalance in the embryo that slows the rate of division; nuclear division would not be synchronized with growth-controlling

substances, and the nuclei are then subject to further deleterious changes. If a mechanism other than genetic imbalance is substituted in this scheme, such as a drastic decrease in division rate from radiation damage to centromeres (or even centrioles in animals) or to a nuclear system not necessarily involving chromosomal aberration, then CAVE and BROWN's hypothesis could explain Type I dominant lethality. A mutant has been discovered recently that, when homozygous in a female Habrobracon, mimics the Type I lethal syndrome in each laid egg (VON BORSTEL, unpublished). Feulgen-negative nuclei that eventually enlarge are produced. It appears that, in the mutant, normal activation of the egg is prevented and the syndrome resembling the Type I lethal embryo is caused by an imbalance of nuclear and cytoplasmic differentiation. This further indicates that the permanent inhibition of mitosis caused by radiation need not be achieved through chromosomal aberration.

Type II dominant lethality: This class of radiation-induced lethality is mimicked in character by the aneuploid proportion of the offspring from Habrobracon translocation heterozygotes. It seems reasonable to conclude that the deaths in each instance have a common origin—chromosomal imbalance. Irradiation of eggs in metaphase I produces chromosome fragments, but no bridges, in anaphase I (A. R. WHITING 1945a,b). Bridges are seen in subsequent divisions, indicating that sister union has occurred and the breakage-fusion-bridge cycle is generated (MCCLINTOCK 1938). Hatchability is lower than would be expected from chromosomal aberrations alone (A. R. WHITING 1945b).

Besides those Type II lethals resulting from the breakage-fusion-bridge cycle, some of the radiation-induced Type II dominant lethals may be caused by formation of a translocation with the subsequent incorporation into the pronucleus of the aneuploid segregant from the resulting heterozygote (MULLER and HERSKOWITZ 1954; PARKER 1954). These are the so-called "half translocations" (ABRAHAMSON, HERSKOWITZ, and MULLER 1956). Other lethals could be caused by formation of translocation dicentrics and still others through production of gross interstitial deletions. It is reasonable to assume that all these could be mimicked in action by the aneuploids from translocation heterozygotes. The proportion of Type II lethals of each kind would, of course, vary markedly with the dose since some would be one hit and others would be two hit. Data presented by HERSKOWITZ and SCHALET (1957) and by PARKER and HAMMOND (1958) indicate that only a small proportion of the dominant lethals induced in Drosophila oocytes could come from translocation formation and aneuploid segregation during the ensuing meiotic divisions.

As the radiation dose to metaphase I eggs increases from 875 to 1100r (Figure 3), the mean of the stage of death of the fertilized eggs is pushed back toward the mean of the stage of death of the unfertilized eggs. This indicates that injury is more severe and that probably several chromosomes become involved. At the higher dose to metaphase I eggs, most of the conditionally delayed dominant lethals are expressed before hatching, but enough still occur after hatching to yield a measurable frequency of conditionally delayed dominant lethality when hatch-

ability is the criterion and the frequency is computed algebraically, whereas none are found at any dose when prophase I eggs are irradiated (Table 5, ATWOOD *et al.* 1956).

Type III dominant lethality: This type of dominant lethality remains somewhat of an enigma. In some respects it would appear to be a more marked form of the Type II dominant lethal—the delayed expression brought about by fertilization of the egg with a normal sperm is still extant but is contracted into the prehatching period of embryogeny (Figure 4). If this is the case, then more chromosomes would have to be involved in bridge-breakage in each embryo than those postulated as occurring in eggs irradiated at 1100r at metaphase I (Figure 3b). However, even at the 1100r dose to metaphase I eggs in which an average of two or more inactivating events per nucleus have occurred, the conditional delay still protrudes into the postembryonic period. Still, if chromatid breakage has a much lower prophase/metaphase sensitivity ratio than Type I lethality or recessive lethal or visible mutations (A. R. WHITING and MURPHY 1955), then Type III lethality could be merely a severe manifestation of Type II lethality. Type III dominant lethality is best mimicked by the aneuploidy resulting from segregation in triploid Habrobracon virgins. This would suggest that loss of one or more chromosomes is brought about by the radiation. It would seem that nothing could mimic chromosome loss better than chromosome loss, but based on chromosome mechanics alone, it is difficult to visualize the origin of such an aneuploid from irradiation of diplotene chromosomes. Mitigating against the hypothesis of chromosome loss accounting for all the Type III lethals is the substantially high proportion of deaths that occur during the larval and pupal stages in unfertilized eggs, which, curiously enough, do not seem to be covered by a normal sperm (Figure 4b). They could be recessive lethals in the haploid that are balanced by conditionally delayed dominant lethals in the diploid. The exact role of recessive lethals in total mortality of unfertilized eggs remains a problem for the future.

The beautiful simplicity inherent in the chromosome imbalance theory of dominant lethality as the primary source of all dominant lethals makes this theory difficult to discard. The theory enjoys an extraordinary advantage in that it is easy to find evidence to support it and nearly impossible to find direct evidence against it. The breakage-fusion-bridge cycle can still be invoked as the source of all the three classes of radiation-induced dominant lethals described here, but restrictions must be included for any postulated type of bridge-breakage that could cause the very early deaths. The principal restriction is that Type I dominant lethals must arise from a specific kind of break that does not get involved in normal chromosomal rearrangement, or that a chromosome breaking during the course of a special type of breakage-fusion-bridge cycle has properties that cause it to block mitosis. Of course, for the mitotic inhibition hypothesis, a mechanism for the mitotic block must be evolved that is compatible with exponential kinetics for sperm or eggs in metaphase I or two-hit kinetics for eggs in prophase I.

CONCLUSION

Although chromosome imbalance phenomena can mimic some of the dominant lethality induced by radiation, the majority of radiation-induced dominant lethals in Habrobracon and Drosophila are not mimicked by genetically contrived loss of chromosomes or loss of chromosome parts.

SUMMARY

1. Three kinds of dominant embryo lethals, distinguished phenotypically, are induced by radiation in Habrobracon; a similar situation exists in Drosophila but only two kinds are distinguished by our criteria.

2. The first type (Type I), and most predominant kind, of dominant lethal is expressed before blastoderm formation in both Habrobracon and Drosophila.

3. The second type (Type II) occurs in Habrobracon and results in death of the haploid embryo after blastoderm formation and before hatching, and predominantly after hatching if the embryo is fertilized with normal sperm. An analogue of this type has not been sought in Drosophila.

4. The third type (Type III) brings about death of the Habrobracon embryo after blastoderm formation and before hatching in either the haploid or diploid embryo; the type of dominant lethal in Drosophila that dies during later embryogeny is analogous since death occurs in the diploid embryo after blastoderm formation but before hatching.

5. Aneuploid eggs from Habrobracon females heterozygous for a translocation mimic the Type II radiation-induced dominant lethals.

6. Aneuploid eggs from Habrobracon triploid females and from Drosophila translocation heterozygotes and triploids mimic Type III dominant lethality.

7. Drosophila zygotes deprived by genetic means of the X and Y chromosomes and lacking the associated nucleoli develop further than radiation-induced Type I dominant lethal embryos.

8. None of the forms of aneuploidy contrived genetically in these experiments mimic the radiation-induced Type I dominant lethal, but a mimicking situation not involving aneuploidy has recently been discovered.

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RANDOMNESS OF MATING IN ISOLATED POLYCROSS PLANTINGS OF MAIZE[1]

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MATING of any naturally outbreeding organism is commonly assumed to be at random in the absence of artificial controls. This assumption is basic to many of the mathematical models used in population genetics. It may have validity for animals, particularly in populations of relatively long standing. In some plants, however, differences in time of maturity of the male and female flowers arising from either morphological pattern or environmental conditions may limit randomness of mating. In corn, for example, a wide variation exists in time of flowering in any open pollinated variety. This variation results in a series of subpopulations, each with some degree of physiological isolation but with opportunity for gene transfer between subpopulations.

Departures from random mating would be of particular importance in new populations that might result from the production of synthetics or the truncated sample from any of the various recurrent selection techniques. Since these methods of breeding are being used extensively, it appeared that information on randomness of mating was of sufficient importance to warrant investigation.

MATERIALS AND METHODS

Ten stocks of maize, each homozygous for a different simply inherited recessive, were planted in three contiguous 10×10 latin squares and allowed to open pollinate. Because of poor stands only 15 successive rows, or one half of the planting, were used in the detailed investigation. One of the stocks used proved to be heterozygous and all plants of it were discarded. Each plot was a single hill consisting of three plants under perfect stand conditions. Notes were taken on an individual plant basis for dates of onset of pollen shedding and of silking, length of pollen-shedding period and plant height. In a separate series of plantings, amounts of pollen shed were estimated by weighing the daily collections of pollen from each plant during the entire pollen-shedding period.

Plants in the latin-square planting were harvested individually. The frequency of selfing or sibbing was obtained directly from the harvested ears when endosperm markers were involved. Self- or sib-pollination in the stocks involving

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seedling markers could be read shortly after seedling emergence. Plants grown from the remaining seedlings were self-pollinated, and each selfed ear was classified to establish the male parentage. Productivity of the nine parental stocks was far from equal. The average number of F_2 progenies per P_1 female ranged from 67 for the lowest to 178 for the highest yielding stock. A total of 11,271 progenies was classified in this study.

The genetic characters used were expressed in either the seed or the young seedling. The seed traits were yellow scutellum, *sy*, waxy combined with an unidentified semisugary type, *wx* su_{205}, sugary, su_1, brittle, bt_1 and shrunken, sh_1. The seedling types were virescent, v_{17}, liguleless, lg_2, white leaf tip, *wt*, and stripe, *str*. The virescent and liguleless stocks each carried an additional linked marker for glossy seedlings, gl_7 and gl_6 respectively. These two glossy seedlings are phentotypically indistinguishable and therefore were not used in the present study.

EXPERIMENTAL RESULTS

The mean values and their standard errors for the various plant measurements are presented in Table 1. The figures for total weight of pollen were obtained by multiplying the total weight per plant in the separate planting by the total number of plants of the same strain in the polycross block. (TYSDAL and CRANDALL (1948) have defined a polycross as "the seed produced on selected clones interpollinated at random in isolation.") The figures are, therefore, approximations. More reliable figures might have been obtained by measuring pollen production directly in the polycrossing block, but this would have vitiated the objective of normal open pollination.

The nine stocks exhibited considerable variation in number of days to silking and pollen shedding. The parental ears of the stocks used were chosen on the basis of similarity in date of pollination. It is obvious that this selection was of limited effectiveness. The selection was on an individual plant basis and may have been

TABLE 1

Means and standard errors for various plant measurements of nine genetic stocks of maize

Stock	Plants shedding pollen (no.)	Length of pollen-shedding period (days)	Total weight of pollen shed (grams)	Days after August 1 for		Plant height (inches)
				Tasseling	Silking	
sy	33	7.5 ± 0.30	8.7	−1.6 ± 0.66	−2.2 ± 0.71	52.9 ± 1.19
wt	36	8.0 ± 0.26	9.5	−1.1 ± 0.68	1.1 ± 0.66	57.9 ± 1.37
gl_7 v_{17}	42	9.2 ± 0.24	9.0	4.3 ± 0.68	7.0 ± 0.17	54.6 ± 1.42
lg_2 gl_6	25	5.5 ± 0.43	11.0	10.5 ± 1.02	12.9 ± 1.15	72.4 ± 1.67
wx su_{205}	17	7.4 ± 0.47	3.4	10.9 ± 1.05	11.6 ± 1.17	75.3 ± 1.59
sh_1	16	7.2 ± 0.49	2.9	11.9 ± 1.23	9.2 ± 1.40	65.4 ± 4.22
su_1	25	7.9 ± 0.38	13.5	13.1 ± 1.25	11.7 ± 1.38	67.9 ± 2.03
bt_1	30	8.1 ± 0.46	41.0	14.5 ± 0.93	15.6 ± 1.58	71.0 ± 2.38
str	35	8.4 ± 0.16	22.5	16.5 ± 0.65	17.5 ± 0.57	58.2 ± 0.46

TABLE 2

Percent of male functioning by the different stocks of maize in the polycross plantings

Stocks	Percent of male functioning by									Total no. F_1's
	sy	*wt*	$gl_7\ v_{17}$	$lg_2\ gl_6$	*wx* su_{205}	sh_1	su_1	bt_1	*str*	
sy	25.4	51.3	17.4	0.2	0.7	0.7	2.6	1.1	0.6	1,405
wt	48.2	17.7	28.0	0.6	0.2	0.9	2.2	1.0	1.2	1,238
$gl_7\ v_{17}$	6.4	7.8	58.1	1.0	2.7	7.2	10.2	5.9	0.7	2,316
$lg_2\ gl_6$	1.6	1.3	9.1	12.4	3.5	12.5	19.2	20.2	20.2	1,424
wx su_{205}	0.7	7.4	19.8	7.3	9.9	14.9	2.8	19.6	17.6	740
sh_1	11.3	4.4	37.8	2.6	3.8	5.9	19.8	8.1	6.3	1,060
su_1	3.6	4.7	23.1	1.2	...	7.3	25.9	10.8	23.4	1,582
bt_1	....	0.9	6.7	5.4	8.4	24.6	8.8	17.0	28.2	687
str	....	0.2	0.4	0.2	...	0.4	21.6	4.5	72.7	819
Total no. F_1's	1,306	1,323	3,030	350	294	825	1,451	1,008	1,684	11,271
Total percent	11.6	11.8	26.9	3.1	2.6	7.3	12.9	8.9	14.9	.....

as effective as can be expected under such conditions. The total range in time of pollen shedding is not appreciably greater than that characteristic of open pollinated varieties of corn.

The percentage frequency with which each stock functioned as either a female or male parent is indicated in Table 2. The stocks are arranged in order of date of pollen shedding. Had mating been at random, each of the nine stocks should have functioned as a pollinator with a frequency of 11.1 percent. The observed percentages range from a low of 2.6 for *wx* su_{205} to a high of 26.9 for $gl_7\ v_{17}$. The agreement between reciprocal combinations was only fair.

The member of a pair having the lower incidence of functioning less frequency as a pollinator was usually the earlier stock. This would be expected, since the period of silk receptivity exceeds the duration of pollen shedding or of pollen viability.

It is obvious that equal functioning of all stocks as males could not be expected because of the significant differences among the strains in dates of anthesis or silking. Such differences would lead to partial or complete physiological isolation depending upon the magnitude of the differences involved. Under such conditions random mating could be shown to have taken place by a demonstration that the pollination frequencies for each stock corresponded to the frequency of the pollen available in the isolated block at the time of silking. An expected number of pollinations was calculated for each plant in the isolated block corresponding to the fraction that the estimated weight of the pollen shed by each stock, during the four days following the emergence of its silks represented of the total weight of pollen produced during the same period. The expectations for the individual plants of a given stock were then combined to obtain a total expectation. A test of significance was calculated from the deviations between observed and expected numbers. The chi-square value exceeded the one percent level of probability for each of the nine stocks. The detailed data were presented by GUTIERREZ (1952) and will not be repeated here.

TABLE 3

Percent of male functioning among five stocks of maize having similar dates of flowering

Stock	Percent male functioning by					Total number F_1's
	lg₂ gl₆	*wx su₂₀₅*	*sh₁*	*su₁*	*bt₁*	
lg₂ gl₆	18.3	5.1	18.4	28.3	29.9	965
wx su₂₀₅	13.4	18.1	27.3	5.2	36.0	403
sh₁	6.4	9.4	14.6	49.4	20.2	425
su₁	2.7	...	16.2	57.2	23.9	715
bt₁	8.4	13.2	38.3	13.6	26.5	441
Total no. F_1's	314	220	635	973	807	2,949
Total percent	10.6	7.5	21.5	33.0	27.4	...

Such calculations may suffer from two limitations: weight of pollen shed in one block may provide a poor estimate of the number and viability of gametes present in the second block, and four days may not be a proper estimate of the period necessary for the completion of all pollination on a given ear after the onset of silking. Limitations of the data available preclude any improvement of the estimate on pollen shedding. LONNQUIST and JUGENHEIMER (1943) reported that under the conditions prevalent in Kansas maximum receptivity of silks from covered shoots occurred two days after first silk emergence.

An alternative approach involves the utilization of five of the nine stocks having similar dates of pollen shedding and of silking. The data from this series are presented in Table 3. Here also the chi-square value for the deviations from 20 percent was highly significant (693.25 with four d.f.). It is necessary to conclude, therefore, that this population of individuals with rather similar flowering habits did not mate at random. Shrunken functioned as a male within reasonable limits of its expectation, *wx su₂₀₅* and *lg₂ gl₆* exhibited negative and *bt₁* and *su₁* positive deviations. In the *lg₂ gl₆* stock the tassels are sparsely branched and anthesis is often initiated while the tassel is still enclosed in the leaf whorl. The deficiency for the *wx su₂₀₅* stock may be related to a slower rate of establishment of the pollen tube, which appears to be characteristic of *wx* type pollen (SPRAGUE 1933). No explanation is apparent for the positive deviations of the *bt₁* and *su₁* stocks.

The interrelationships of number of plants shedding pollen, length of pollen-shedding period, weight of pollen shed, plant height, date of tasseling and date of silking with the over-all performance of the stocks as male parents were examined by means of regression analyses (Table 4).

The regression of percent of male functioning on number of plants shedding pollen was positive and significant. It accounted for 64.1 percent of the variation in male functioning. Similarly, the regression involving length of the pollen-sheddng period accounted for 65.6 percent of the variation in percent of male functioning.

A strong association would be expected to exist between number of pollen grains and percent functioning. Such a relationship was not apparent. The lack

TABLE 4

Regression analyses of number of plants shedding pollen, length of pollen-shedding period, weight of pollen shed, plant height, dates of tasseling and silking on percent functioning as male parent in maize stocks

Attribute	Source of variation			
	Regression (1 d.f.)	Mean square deviation from regression (7 d.f.)	F value	b
Number of plants shedding pollen	271.91	21.67	12.55**	0.66
Length of pollen-shedding period	279.27	20.62	13.54**	5.81
Weight of pollen shed	1.70	60.27	....	0.04
Plant height	233.50	27.16	8.60*	—0.65
Date of tasseling	4.28	59.90	....	0.11
Date of silking	1.60	60.28	....	0.07

** Significant at the 1 percent level.
* Significant at the 5 percent level.

of relationship suggests either that weight as determined provided a poor estimate of numbers or that a high degree of selective fertilization was involved. Dates of pollen shedding and silking did not appear to be significantly related to percent of male functioning. The apparent lack of significance for these variables merits further consideration. It will be noticed (Table 2) that the higher values for male functioning tend to occur along the diagonal, indicating a tendency toward sibbing and crossing among individuals of the same maturity group. Under such conditions the association of both date of pollen shedding and date of silking with percent of male functioning would be expected to vary with maturity group. The intra-stock correlations between percent of male functioning and either date of pollen shedding or date of silking were negative in the earlier stocks, near zero for those of intermediate maturity and positive for the later group. These variations in trend account for the over-all association being essentially zero.

Of the several characters measured, number of plants shedding pollen, length of pollen-shedding period and plant height were found to be significantly associated with percent of male functioning. These associations were examined further by the partial correlation analysis presented in Table 5. When the linear effects of either length of pollen-shedding period and plant height or both were held constant, the partial correlation between number of plants shedding pollen and percent of male functioning was nonsignificant. In contrast, the correlation between length of pollen-shedding period and percent of male functioning was independent of the number of plants shedding pollen and of plant height.

Each of the variables discussed so far appears to have some relation to the departures from randomness of mating. However, singly or in combination they do not provide a satisfactory explanation for the deviations observed. Data are available on two additional items which might influence randomness of mating: position in the polycross block and selective fertilization. Since the original planting arrangement was in the form of three contiguous 10×10 latin squares, the possibility of an important location effect was reduced. Nevertheless a correlation was calculated between the number of times two given stocks were adja-

TABLE 5

Simple and partial correlation coefficients between number of plants shedding pollen (n), length of pollen-shedding period (l), plant height (h) and percent of male functioning (p) in maize stocks

Simple correlation coefficients		Partial correlation coefficients			
		First order		Second order	
r_{np}	0.80**	$r_{np.l}$	0.66	$r_{np.lh}$	0.44
		$r_{np.h}$	0.54		
r_{lp}	0.81**	$r_{lp.n}$	0.68	$r_{lp.rh}$	0.80
		$r_{lp.h}$	0.72*		
r_{hp}	−0.74*	$r_{hp.n}$	−0.34	$r_{hp.nl}$	−0.32
		$r_{hp.l}$	−0.60		

cent and the percent of cross-pollination between the same two stocks. The correlation was −0.178 and nonsignificant. Therefore position appeared not to be an important factor influencing departures from randomness of mating.

JONES (1924) demonstrated that selective fertilization may be of importance in pollen mixtures and that in certain cases there is a consistent tendency for increased receptivity of a plant to its own pollen. This tendency plus the differences in maturity may account for the high values of male functioning of each of the stocks on the parental type.

A number of reciprocal crosses were made involving seven of the nine stocks and using pollen mixtures to detect differential pollen functioning. The kernels obtained were classified into four groups: A × A, B × B, A × B, and B × A. The A × A and B × B groups represent functioning of the pollen produced by the stock being pollinated, and the A × B and B × A groups represent the functioning of the complementary pollen of the mixture. The chi-square values obtained in comparing relative rates of pollen functioning were significant in the majority of cases. However, the results do not contribute greatly to an understanding of departures from random mating. There was a general failure of agreement between independent comparisons involving any two given stocks. It was possible to detect a slight trend toward decreased functioning of *sy* pollen in selfing and of *su* in cross combinations when in mixtures. Neither of these two tendencies, however, would explain the departures from random mating observed in the polycross planting. Corn pollen is difficult to handle in bulk, and it may well be that differential clumping was partially responsible for the failure of agreement between the different independent pollen mixtures.

DISCUSSION

The data obtained in this study indicate that random mating was not realized under the open pollination conditions prevailing. There was a marked tendency for an excess of selfing or sibbing and for less than expected cross-pollination between strains of similar maturity. Consideration was given to following mating in a second cycle of open pollination, but space and facilities would not permit. The question remains, therefore, as to whether a closer approach to randomness

would have been attained in the next cycle of open pollination. It would appear that the same factors operating in the present study would have persisted to a greater or lesser extent through at least the next cycle of open pollination.

One of the major factors involved in the departure from random mating in this study was difference in time of flowering. In the second cycle of open pollination such differences would no doubt be equally great if the parental seed was a bulked sample from the isolated planting. The individuals comprising this bulk would have resulted from self- or sib-pollination and from crossing. Because of inbreeding depression effects, selfing or sibbing would result in some delay in time of flowering, whereas hybridization would tend to shorten the period from emergence to flowering. These changes might facilitate crossing of hybrids among the later strains with self or sibbed individuals from the earlier types. Selfed individuals from the later flowering strains would, however, be even more effectively isolated than in the original planting. Because of differences in seed yields involved (Table 2), such types might tend either to be eliminated from the population or to persist in reduced frequency.

The attributes plant height, number of plants shedding pollen and length of pollen-shedding period would be expected to exhibit some degree of heterosis. This would have some influence on the disparity of functioning of the inbred versus hybrid material. It would appear, therefore, that several open pollination generations might be required before the establishment of equilibrium. Furthermore, the gene frequencies at equilibrium might have little relation to the random-mating expectations for the initial set of nine marked stocks.

In situations where it is desirable to perpetuate the average gene frequency of a selected group of tested individuals, as in the production of a synthetic or the truncated sample from a recurrent selection series, the departures from randomness of mating can be minimized if equal quantities of the $\frac{n\,(n-1)}{2}$ possible crosses are bulked rather than through the bulking of equal numbers or quantities of the original stocks. Selective elimination following such a procedure would presumably be due to variation in fitness rather than partial isolation arising from differences in time of flowering or the amount and type of seed produced.

If extrapolation from the present study to open pollinated varieties of corn in general is permissible, it would appear that random mating, in the strict sense, may not be realized. Such failure could result from variation in time of pollen shedding and silking, some degree of selective fertilization and genotype-environment interactions.

The range in times of pollen shedding and silking for plants comprising an open pollinated variety may exceed 14 days (KIESSELBACH 1922). In relatively unselected varieties this time period may be much greater. Under such conditions there would be a strong tendency within the early group for pollination to be restricted to this group. The same situation would prevail within the late group. The groups of intermediate flowering time would be less effectively isolated. Crossing would occur between plants within such groups and to a lesser extent between groups.

The data on selective fertilization involving pollen mixtures was inconclusive. However, since mating was not random (Table 3), even when differences in time of flowering had been minimized, it appears that some type or degree of selective fertilization must have been involved. To the extent that this phenomenon was of importance, it would tend toward stratification rather than homogeneity.

The productivity of the early, mid-season and late-flowering types within a variety and the inbred *vs.* hybrid groups would be expected to vary from season to season because of genotype environment interactions. SPRAGUE and FEDERER (1951) demonstrated that the variance components for genotype-environment interactions for yield may be of considerable importance in replicated single-cross yield trials. Observational data suggest that the same situation obtains for time of flowering. If such results obtain to an equal degree for the genotypes comprising an open pollinated variety, then individual contributions to the following generations would be expected to exhibit considerable fluctuations. It would appear there are involved two distinct sets of forces, one leading to some degree of stratification and the second permitting some degree of recombination among the strata. The net effect may be to maintain a high degree of genetic variation rather than an orderly progression toward a stable equilibrium.

SUMMARY

Polycross plantings involving genetically marked stocks of maize exhibited marked departures from random mating.

Dates of silking and pollen shedding, number of plants shedding pollen, length of the pollen-shedding period and plant height were shown to be associated with the observed departures from randomness.

The possible bearing of these findings on breeding procedures and on equilibrium values in open pollinated varieties of maize is discussed.

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CYTOGENETICS OF A TRISOMIC OF MATTHIOLA INCANA, INVOLVING A RING CHROMOSOME AND SOMATIC INSTABILITY OF SINGLENESS (VERSUS DOUBLENESS) OF FLOWERS AND SHAPE OF LEAVES[1]

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DOUBLE Matthiola flowers are completely sterile, since they consist of masses of indefinitely replicated petals. Double-flowered plants can be obtained only from seed of singles that are heterozygous (*Ss*) for doubleness, which is recessive to singleness. Any ordinary (diploid) floricultural variety consists of slightly more than 50 percent sterile doubles and slightly fewer fertile singles (SAUNDERS 1911). The single line is kept permanently heterozygous for doubleness (eversporting or double-thrower) by a pollen lethal, *l*, which sterilizes the single-carrying or *Sl* pollen of *Sl/sL* plants and slightly reduces the viability of the *Sl* embryo sacs. This lethal is occasionally lost, in some races at least (GOLDSCHMIDT 1913; FROST 1915; FISHER 1933; KAPPERT 1951). The genetics of floral doubleness in Matthiola diploids has been extensively reviewed by KAPPERT (1951) and by JOHNSON (1953). A brief review has been presented by FROST and LESLEY (1954), introductory to a popular account of the floriculturally useful Slender trisomic, whose seed gives exceptionally high progeny proportions of double-flowered plants.

MATERIAL AND METHODS

The Slender trisomic

The Slender trisomic has been found several times (FROST 1919) in progenies of normal appearing (doubtless diploid) plants of Snowflake, a small, early, glabrous, white-flowered, double-thrower variety which differs from other varieties in having exceptionally elongated chromosomes that occasionally stretch and break at first anaphase of meiosis (LESLEY and FROST 1927). It is therefore not surprising that Snowflake produces occasional plants with extra chromosomes or chromosome fragments. Diploid *Matthiola incana* has seven pairs of chromosomes.

Three forms with comparatively large extra chromosomes (Crenate, Narrow, and Dark) may be primary trisomics, each carrying three complete homologous chromosomes. Slender and two other extra chromosome types out of a

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total of nine produced by Snowflake have been shown to be fragment secondaries (FROST 1919; FROST and MANN 1924; LESLEY and FROST 1927, 1928; FROST 1927, 1928, 1931).

The Slender trisomic (FROST 1919, 1927) is characterized by slenderness of stems, leaves, and capsules, which is most conspicuous in the relatively narrow, long-petioled leaves and the drooping racemes. The extra chromosome of Slender has been shown to be a rod-shaped fragment approximately half as large as one of the two homologues. This fragment has been transmitted by as much as 18 percent of the pollen (FROST 1928; normal by Slender) and more extensively by the ovules. The fragment cannot replace a whole chromosome. The centromere of the fragment is at or near one end, that of Crenate approximately central. In meiosis the fragment is often lost, but in mitosis it is usually divided normally. In the double trisomic Crenate Slender, the fragment chromosome of Slender often forms bivalents with one of the three large homologous chromosomes of Crenate (FROST 1931). The Crenate trisomic has given progeny ratios (FROST 1931, Table 1) showing unquestionably that its extra chromosome carries the single-double (*S*) locus. Genetic evidence demonstrates that the fragment chromosome of Slender also carries the *S* locus. Another secondary, Crenatoid, has been identified only among offspring of Crenate parents. Crenatoid considerably resembles Crenate in leaf shape, but both are very unlike Slender. The extra chromosome looks like that of Crenate and, like it, is usually V-shaped. ARMSTRONG and HUSKINS (1934) suggested that Crenatoid may have one limb of the Crenate chromosome duplicated. Selfed double-thrower Crenatoid gave disomic ratios for doubleness, therefore it presumably lacked the *S* locus.

In the earlier genetic studies with the Snowflake variety, the tested *S/s* singles regularly indicated presence of the closely linked lethal *l*. Singleness is dominant in *ssS* plants, *sss* giving doubles. It is assumed in this discussion that the *Sl* combination of diploid Snowflake (*sL/Sl*) was regularly retained by the derived single Slender Snowflake trisomics. It must be noted however, that such an *ssS* trisomic without the lethal presumably would give partly similar progeny ratios, as long as the *S* was in the fragment chromosome. If *l* was present, some evidence indicates that functional pollen can carry *Sl* if it also has a homologue carrying *sL* (FROST 1928). Since Snowflake Slender trisomic parents regularly gave progeny ratios that indicated the presence of only one *S* gene, it is probable that two *Sl* chromosomes cannot be carried by a viable trisomic Slender zygote.

With the *S* and *l* factors in the fragment chromosome, the composition may be represented by *sL/sL/(Sl)*, the parenthesis indicating the fragment. Such trisomics should give, with selfing, only diploid double and trisomic single offspring, and occasional tetrasomics—except as a result of crossing over between the fragment and one of the homologous whole chromosomes. Since the lethal *l* (KAPPERT 1951) and the extra fragment chromosome both decrease viability, a large excess of diploid doubles, *sL/sL*, commonly results (Table 1, parents 1–3). For the first of these three parents, the genotype was convincingly shown

TABLE 1

Percentages of doubles and trisomics in progenies from selfing of trisomic Slender

Parent		Progeny*				
		Percent double				
Number	Formula	of diploids	of trisomic Slenders	of all plants	Slender, percent of all plants	Number of plants
Parents: Snowflake double-thrower; trisomic Slender selfed						
1	*sL/sL/(Sl)*	94.2	19.0	72.6	28.8	146
2	Same	98.1	7.1	67.1	34.1	82
3	Same	96.1	25.0	76.6	27.4	175
1 to 3	Same	95.8	18.6	73.2	29.3	403
4 to 7	*Sl/sL/(sL)*?	72.9	25.2	51.3	45.3	236
Parents: F_1 from pure-single variety, *SL/SL*, and trisomic Slender double-thrower (Snowflake), *sL/sL/(Sl)* or *Sl/sL/(sL)*†						
8	*SL/sL/(sL)*	21.5	27.1	23.7	38.8	152
9	Same	25.5	25.0	25.3	42.0	162
8 and 9	Same	23.5	26.0	24.5	40.4	314
10	*SL/sL/(Sl)*	28.6	0	21.1	35.2	142
11	*SL/Sl/(sL)*?	1.4	0.8	1.1	49.4	271

* These percentages are calculated from the numbers of plants published by FROST (1931, Table 2). These results seem typical; others are unpublished.
† Slender parent 2 was male parent of parents 8 and 10, and female parent of parent 9.

by a seedless variant branch; partial change to double flowers and normal leaves indicated loss of a fragment carrying the only *S* gene (FROST 1926, p. 42).

F_2 progenies from crossing of pure-single pollen-fertile diploids, *SL/SL*, with trisomic double-thrower Slender, indicated that the extra chromosome gametes had usually carried either *sL/(Sl)* or *sL/(sL)*. F_1 parents 8 and 9 of Table 1 gave about 25 percent doubles in F_2 among both diploids and trisomics, hence F_1 must have been *SL/sL/(sL)*, without the lethal. F_1 parent 10, in contrast, produced no doubles among the F_2 trisomics and therefore was *SL/sL/(Sl)*. One F_1 plant tested, parent 11, produced very few F_2 doubles, presumably only through meeting of crossover gametes; it may have been *SL/Sl/(sL)*, having received *Sl/(sL)* from its trisomic parent. With this constitution, every viable noncrossover gamete must have a whole chromosome carrying the dominant *S*.

For such ratios as those from parents 4 to 7 (Table 1), aside from the possibility of mere sampling differences, there is some evidence (from tests of F_2 hybrids with pure single) indicating that these ratios may result when *Sl* is in the fragment chromosome, and also evidence (from frequent transmission of *s* fragments to F_1 hybrids) that the suggested *S/s/(s)* constitution probably occurred among trisomic parents.

Plant types and cultures

In 1953 LOCKE found one plant that had both single and double flowers. The Slender parent of this variant was a descendant of Snowflake Slender, through

a succession of crosses with colored-flowered double-thrower races. He has grown and studied four generations of descendants of the variant Unstable plant at Guadalupe. California. The data from those cultures that are presented here were mainly recorded by the Riverside authors late in the period of full bloom. Three generations have been grown at Riverside, on a smaller scale, by FROST; these cultures have furnished material, from both field-grown and potted plants, for cytological studies by M. M. LESLEY.

In both localities, the tested parents have included not only Unstable plants, but also trisomic Slenders and diploids with only single flowers, which had appeared among offspring of Unstables. Since Matthiola is mainly self-pollinated, flowers were not bagged for seed production in this study. Probably the results were not seriously affected by cross-pollination. Two progenies at Guadalupe in 1956 and 1957 had varied flower colors, indicating that the parents had resulted from cross-pollination from outside the study of Unstable. Otherwise, the flower color (dominant purple) was very uniform. Plant type, as represented by diploids, was also conspicuously uniform.

At Riverside, the cultures were usually small. At Guadalupe in 1957, few progenies contained fewer than 45 plants or more than 72; in 1956, nearly all progenies had from 35 to 48 plants.

A very few plants had extra chromosome characters not derived from the single-double chromosome pair. Such characters were ignored in tabulation of trisomics and diploids.

Cytological methods

The pollen mother cells of all plants having both single and double or only single flowers were studied in progeny 55.1, and those of the majority of such plants in 56.F1 to F5 from parents in 55.1 (Table 2). P.m.c. from trisomic

TABLE 2

Progenies of trisomic Unstable (Riverside)

Progeny number	Parent		Progeny‡							
	Generation of Unstable	Origin	No. of seeds sown	No. of plants	Percent double	Diploid		Unstable, flowers mixed	Slender	
						Sg	Db		Sg	Db
55.1	2	Guad. 1954	90	85	74	0	63	20*	2	0
56.F1	3	55.1–1	50	35	71	0	25	10	0	0
56.F2	3	55.1–7	50	42	67	1	27	13	0	1
56.F3	3	55.1–15	50	46	67	0	31	15	0	0
56.F4	3	55.1–44	50	32	81	0	26	5	1	0
56.F5	3	55.1–63	30	19	95	0	18	1	0	0
56.F7	3	55.1–16	50	50	30	34?	15	1†	0	0
56.F8	4	56.5–9	50	38	87	0	33	5	0	0
56.F9	4	56.7–9	50	14	71	0	10	4	0	0
56.F10	4	56.8–4	50	41	78	0	32	9	0	0

* One of these 20 plants, 55.1–16, was Unstable in leaf characters, but its flowers were all single.
† This plant and some listed as single had slight or very slight floral abnormality. (See text).
‡ Sg = single, Db = double.

Slender plants of 56.F11 and F12 were also studied. Orcein smears were used in this general survey. Anthers from selected plants were fixed in acetic-alcohol and smeared by Walter's modification of the aceto-carmine method (BROWN 1949). Mitosis was studied in premeiotic and tapetum cells found in these smears, and in sections of root tips fixed in chrom-acetic and stained in gentian violet.

THE UNSTABLE TRISOMIC

Somatic variation in offspring of trisomic Slender, to or toward diploid double, in a plant that had been trisomic single, has been observed several times (FROST 1926 and unpublished). Loss of the extra fragment chromosome in a meristematic cell, with chimera formation, was postulated in explanation. The only progeny test of such a plant (Table 1, parent 1) showed that its slender main portion was ordinary (stable) Slender. The recently discovered Unstable trisomic typically has leaves varying from slender to diploid-type, and flowers varying from single to fully double. The present study is an attempt to explain the irregular inheritance and expression of the new type, which has a somatically unstable extra chromosome that is usually a ring.

Somatic characters of Unstable: In leaf shape, Unstable trisomics commonly present very irregular mosaics of diploid, trisomic Slender, and intermediate characteristics. The leaves are often unsymmetrical, twisted, and rough-surfaced. Figure 1 shows primary leaves of Unstable, Slender, and diploid plants developed under especially favorable greenhouse conditions.

The flowers usually constitute extremely variable mosaics of single, double, and intermediate conditions. Some racemes may have only, or mainly, normal single flowers; more often some racemes are completely double, and very many have flowers that are in some degree intermediate, with or without one or more doubles. The intermediate flowers range from nearly typical single to nearly typical double, with great variability in number of petals and in development of stamens if these are present (Figure 2). Some flowers are almost fully double but have a sterile pistil. One plant was completely double except for scattered flowers with sterile pistils. The slightest noticeable indications of abnormality are the presence of one or more extra petals (often very small) and curvature of the pistil. Most partially double flowers have few p.m.c. Pollen may be present or absent. Probably the most peculiar feature is the occasional occurrence of "inside-out" flowers, in which a sterile silique has an inflated wall and a proliferating axis inside which produces a bunch of petals that sometimes bursts the wall. The pistils produce normal or bent seed-bearing capsules, or variable sterile development, slender or inflated, or they even abort completely at the flowering stage.

Any of these conditions may occur together on one raceme; even one double flower may be present in the midst of seed-filled capsules (Figure 3). Occasionally, and in two progenies (56.F1 and F3, Table 2) frequently, a plant was described before flowering, without question, as diploid in leaf type, but later was found to have mixed flowers.

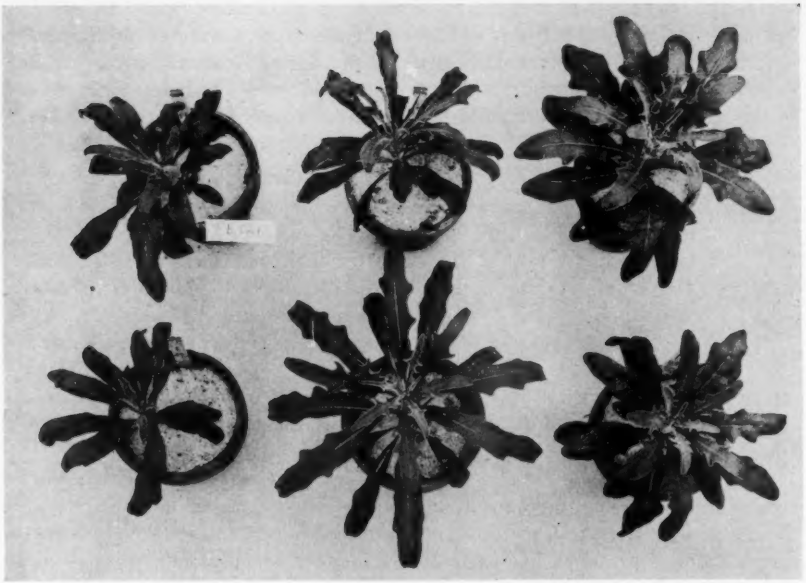


FIGURE 1.—Plants of progeny 55.1 (see Table 2); parent, 1954 Unstable, from seed of Unstable "mutant." *Upper row* (left to right from label): 55.1–16 (Unstable trisomic) leaves mixed, flowers single, $SL/sL/(sL)^{0}$; 55.1–7 (typical Unstable trisomic) leaves and flowers mixed, $sL/sL/(Sl)^{0}$; 55.1–25 (diploid) flowers double. *Lower row:* 55.1–39 (Unstable) like 1–7; 55.1–66 (Slender trisomic) flowers single, 55.1–49 (diploid) flowers double.

Assuming presence of the lethal, the usual type of Unstable described above, may be represented by the formula $sL/sL/(Sl)^{\circ}$, in which the circle indicates the ring form of the extra chromosome. This constitution is to be understood in the following presentation, except where the constitution with *S* in a whole chromosome is especially considered.

Cytology of Unstable: It was stated above that most of the Unstable plants that we have studied are trisomics with a ring-shaped extra chromosome. This ring is lost at irregular intervals in somatic divisions. When the plant is $sL/sL/(Sl)^{\circ}$, loss of the ring gives rise to diploid sL/sL cells that cause the development of double flowers and normal leaves. A plant which was originally Slender single thus becomes variegated for flower and leaf type. Such a chimerical combination of $sL/sL/(Sl)^{\circ}$ and sL/sL tissues may produce shoots with double, single, or intermediate flowers, or various combinations of these as has been described above. It is apparently a matter of chance when and how often the ring is lost in the soma, and how the resulting chimeras develop.

The ring form of the extra chromosome can be determined with certainty in mitotic prophase (Figure 4) or metaphase, at pachytene and occasionally at

FIGURE 2.—Plant 55.1–7. (Unstable trisomic) parent of progeny 56.F2, *sL/sL/(Sl)*°. Separate flowers, ranging from nearly normal single (at left) to nearly normal double (at right).

diplotene. The extra chromosome often lies at one side of the group at pachytene or MI, indicating that crossing over with a normal homologue has not occurred. When crossing over has occurred, the ring is often attached to both homologues at pachytene. Such crossovers lead to the formation of pseudobridges, in which the normal homologues are already separated, while the ring remains attached to them and divides at the equator (Figure 5). It is thought that this is partly the result of the fact, clearly shown by all unpaired chromosomes in meiosis, that division is slower than separation. It may also be that ring-shaped elements, especially large ones containing a duplication, tend to divide even more slowly than rods. Crossing over or twisting may also complicate separation.

The structure of the large ring at pachytene indicates that it consists of two similar parts (Figure 6). These two parts may appear to be paired in early pachytene. The homologous chromosomes have a median centromere and are quite unlike the nucleolar pair which PHILP and HUSKINS (1931) believed to be the *S*-bearing chromosomes (Figures 7a and b).

The ring chromosome usually divides equally and completely, but occasionally a dicentric ring is found both in mitosis and in meiosis (Figures 8 and 9). Such rings must break in anaphase or remain intact and be lost. As a result of breakage, extra rings and rods of various sizes are found. One anther may contain cells with extra chromosomes of several sizes and shapes (Figure 10). Usually, a group of p.m.c. has one type of extra chromosome, showing that breakage must have

FIGURE 3.—Plant 56.F3–35 (Unstable trisomic). Three branches; (left) mainly good capsules, some sterile capsules, one double flower; (right) aborted pistils below, sterile petal-filled capsules and double flowers above; (middle) complete range from nearly normal capsules to double flowers.

occurred in a somatic cell. A single cell or a small group of cells may have two extra fragments of the same or of different sizes. Unstable plants have been shown to differ in the proportion of large, medium, and small extra chromosomes that are typically present. The behavior of the ring in Matthiola is essentially like that described by McCLINTOCK (1938).

Genetic behavior of Unstable and derived types: Progenies of trisomic Unstable usually consist mainly of a minority of trisomics having mixed flowers, and a majority of completely double-flowered plants with diploid characters

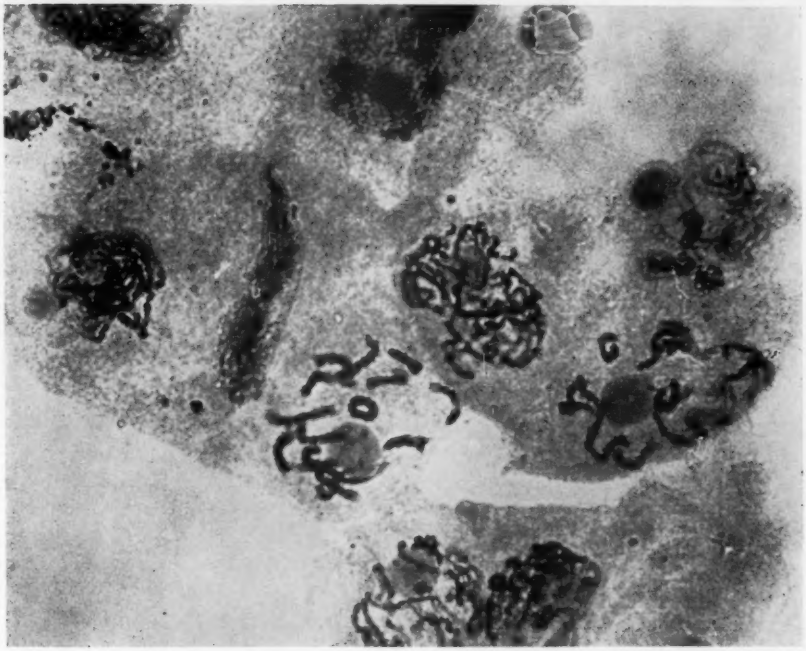


FIGURE 4.—Photomicrograph of a premeiotic cell in late prophase, showing a large ring and 14 chromosomes.

(Tables 2 and 3). Two completely single progeny types have often occurred, usually in very small proportions: trisomic Slender and diploid. Slender doubles have been scarce; in Table 3 they are not reported separately from diploid doubles.

In small progenies from Unstable parents, the percentage belonging to the Unstable type was nearly always much smaller than the percentage having exclusively double flowers. The proportion of double plants was usually above 65 percent, and often exceeded 85 percent. In general it has seemed to be typically higher than in progenies of double-thrower Slender.

The parent of progeny 55.1 (Table 2) was an offspring of the original Unstable variant. In this progeny, 19 of 85 plants were Unstable with mixed flowers, one appearing to be tetrasomic. Three plants had only single flowers; one of these (55.1–16; Figure 1) was clearly Unstable in leaf form; one (55.1–66; Figure 1) had Slender-type primary leaves, and seemed to be ordinary Slender; the third (55.1–58) was described as Slender, although some young primary leaves seemed diploid. Two Unstable plants (55.1–33 and 48) were double trisomics, showing leaf characters of the Narrow and the Dark trisomic, respectively. The rest of the plants in 55.1 were double flowered and appeared to be diploid.

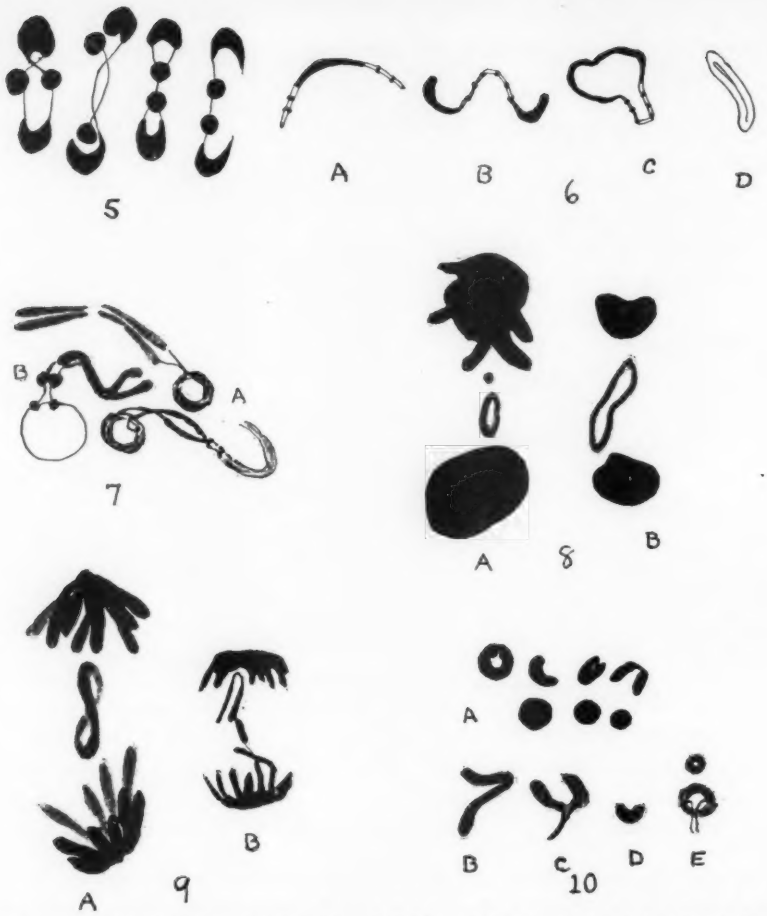


FIGURE 5.—Pseudobridges showing delayed division of the ring and twisting in trivalents at AI.

FIGURE 6.—The large ring at pachytene: (a) broken in euchromatin; (b) broken in heterochromatin; (c) unbroken; (d) unbroken, paired or collapsed.

FIGURE 7.—(a) The *S* chromosome attached to the large ring at pachytene and diplotene. (b) Nucleolar chromosome at diplotene.

FIGURE 8.—(a) AI in p.m.c. showing a lagging ring and fragment. (b) AII in p.m.c., showing a lagging dicentric ring.

FIGURE 9.—(a) Premeiotic cell showing lagging dicentric ring. (b) Root tip cell with double bridge and fragment.

FIGURE 10.—(a) Above: ring and V-shaped extra chromosomes at pachytene. Below: small, medium, and large extra ring chromosomes at MI. (b) Uneven extra V at pachytene. (c) Broken ring at pachytene. (d) Small V at pachytene. (e) Small and medium ring at pachytene.

All drawings were drawn with a #20 ocular and oil immersion; magnification of drawings 3100 diameters.

TABLE 3

*Progenies of trisomic Unstable with mixed flowers, in 1957 (Guadalupe)**

Percentage of plants with only double flowers											
Class (%):	52.4	57.4	62.4	67.4	72.4	77.4	82.4	87.4	92.4	Total	Mean percent
Progenies	1	1	1	9	12	17	20	12	5	78	78.5
Percentage of Unstable trisomics with mixed flowers											
Class (%):	2.4	7.4	12.4	17.4	22.4	27.4	32.4	37.4		Total	Mean percent
Progenies	1	9	17	21	14	12	3	1		78	18.4
Number of diploids with only single flowers											
Class:	0	1	2	3	4	5	6	10	11	12	Total	Mean diploid singles percent
Progenies	43	11	11	5	1	1	2	1	1	1	77	2.1
Number of Slender trisomics with only single flowers											
Class:		0		1		2		3		Total	Mean Slender singles percent
Progenies		55		15		4		3		77	0.7

* Percentages in last column are based on grand totals of plants. In 1956, Unstable parents gave similar distributions for these classes, with the last two not separated.

In the fall of 1956, progenies (designated 56.F) from plants in 55.1 that had, most commonly, either a large ring, an intermediate-sized ring or rod, or a very small ring or rod, in addition to the normal complement, were grown to determine the type of segregation that occurred, the effect of ring or rod shape on variegation, and the effect of different fractions of the ring on transmission of characters.

In plant 55.1–7 many p.m.c. had a large ring. This ring appeared to be composed of two similar parts joined at both ends (Figure 6c). Other p.m.c. had medium or small extra chromosomes. As in other plants with a large extra ring in many p.m.c., bridges occurred commonly in meiosis. Thus breakage and probably also partial or complete nonreduction might be expected to occur in meiosis. Similar bridges also occurred in mitosis (Figures 8 and 9). The progeny of plant 55.1–7, like progeny 55.1 itself, was exceptionally varied. Two plants were extremely small; one of these was triploid, the other trisomic. One plant was very compact with many thick slender leaves. It was described as apparently tetrasomic Unstable; it had a large ring in some p.m.c. and two medium-sized rings in others. The Unstable plants were of very different types. When a large ring is present commonly in the p.m.c. of the parent, a considerable variety of different fragments with a centromere occur, and the trisomic progeny therefore presents a variety of types.

In several plants of 55.1 few large rings were found. The parent of 56.F3 was of this type. Only one plant in 56.F3 had a large extra chromosome; the rest of the trisomics had intermediate rings. The Unstable plants were similar in type; single-flowered branches occurred rarely, and intermediate and double flowers were closely intermingled over most of the plant. The leaf shape often appeared diploid.

Although 55.1–1 had both single and double flowers, its primary leaves were normal in appearance, and the plant was classified as diploid until it flowered.

Most of the p.m.c. had a small extra chromosome, and this was found to be rod-shaped at mitotic metaphase (Figure 11). It appears to have a central constriction. Rings were rarely found and few meiotic bridges occurred; instead, the extra chromosome often lagged and was lost. The Unstable plants in the progeny of 55.1–1 (56.F1) also had nearly normal leaves. The plants were vigorous, and the branches with mainly single flowers were conspicuous. All of the Unstable plants examined in this family had a small rod-shaped extra chromosome.

Out of 98 clear somatic metaphases in 56.F1–29, 69 had 14 chromosomes and one small rod, 27 had only the normal complement, and 2 had 14 chromosomes and two small rods. The loss of the rod seems, in this case, clearly responsible for somatic variegation. In the sibs of 56.F1–29 similar somatic cells were commonly found. It should, however, be mentioned that rarely tapetal cells in 56.F1–29 had a typical large ring in addition to 14 chromosomes and 1 or 2 small rods (Figure 12).

The floral instability of 55.1–1 and of its progeny (56.F1) indicates that *S* was present in the extra chromosome. Since *S* was in this small fragment, its locus appears to have been near the centromere. The tendency to diploid leaf shape in these trisomics indicates that a portion of the extra chromosome located farther from the centromere carried the gene or genes for slenderness.

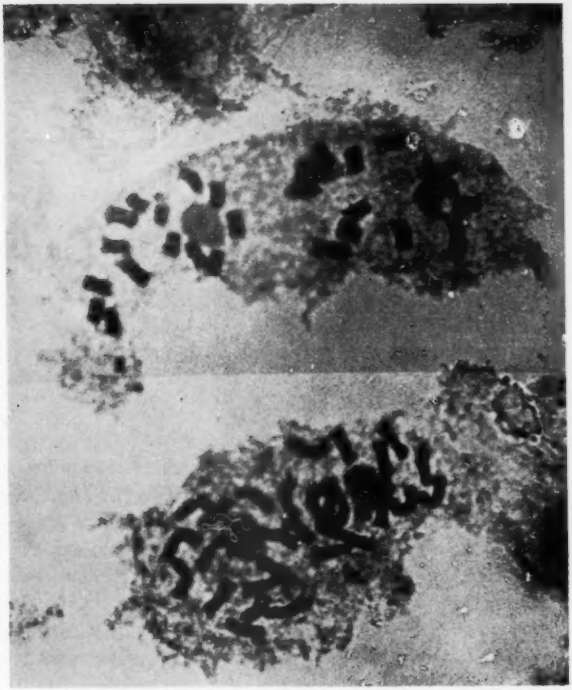
One trisomic greenhouse plant (55.1–16; Figure 1) which produced only single flowers, had unquestionably mosaic leaf characteristics. This plant produced an exceptional progeny (Table 2, 56.F7). No plants were recognized as Unstable or Slender by leaf characters. Of 50 plants, 15 were completely double, and 35 were single except for rare flowers on some plants, with one or rarely several extra petals. One plant had several bent capsules. One plant, examined cytologically because of a very slight flower abnormality, had a large extra chromosome. The three other plants examined had the diploid number. The progeny ratio, approximating 3 single:1 double, indicated that the *S* gene was present in a whole chromosome without the lethal *l*; the parental constitution probably was $SL/sL/(sL)^{\circ}$. Thus somatic loss of the extra chromosome would not result in loss of the singleness gene.

Table 3 includes frequencies of single-flowered diploids from Unstable parents. Some such variants were tested. In 1956, at Guadalupe, 19 parents, offspring of Unstable, described as single diploid, gave progenies, not counted, that had a great excess of single-flowered plants. At Riverside (Table 4, progenies 56.1 and 56.9), two first-generation diploids gave diploid progenies totaling 46

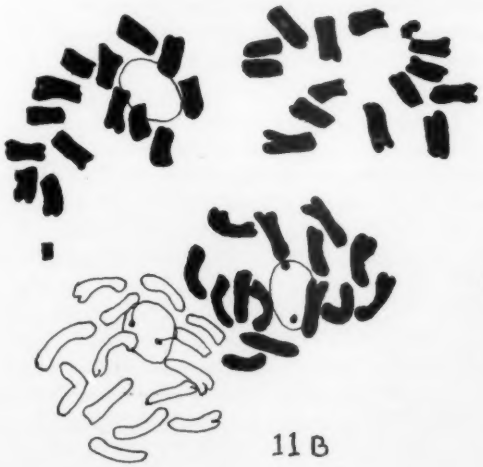
FIGURE 11.—(a) Photomicrograph from 56.F1–29, showing two adjacent binuclear tapetum cells; (b) a drawing of the same. Below, diploid nuclei (note two satellites on nucleolus at left); the cell above has 14 chromosomes and a small rod-shaped extra chromosome in each nucleus.

FIGURE 12.—An exceptional tapetal cell from 56.F1–29 with 14 chromosomes, 2 fragments, and a ring.

FIGURES 11b and 12 were drawn and the photomicrographs were taken with a #6 ocular and oil immersion; magnification of drawings 1300 diameters.



11 A



11 B

12.

TABLE 4

Progenies of single diploid extracted from trisomic Unstable (Riverside)

Progeny number	Parent		Progeny				
	Generation of diploid	Origin	No. of seeds sown	No. of plants	Percent double	Diploid	
						Single	Double
56.1	1	Guad. 1955	30	24	8	22	2
56.9	1	Guad. 1955	30	27	11	24	3
56.F16	2	56.1–2	50	41	22	32	9
56.F17	2	56.1–18	50	39	28	28	11
56.F18	2	56.9–7	50	38	29	27	11
56.F19	2	56.9–20†	50	44	0	44	0
56.F20	2	56.9–26	50	41	22	32	9
56.F21	1	56.2–22*	50	44	23	34	10
56.F22	1	56.3–10*	50	45	24	34	11

* These two diploid plants (56.2–22 and 56.3–10) occurred in the first two progenies of Table 5. They were therefore offspring of single Slenders, the latter being offspring of Unstable, and were sibs of the last two parents of Table 5.
† A later culture from parent 56.9–20 consisted of 82 plants, all single.

singles and 5 doubles; four of these (second-generation diploids) gave progenies closely approaching 25 percent doubles (56.F16, F17, F18, and F20), and one (F19) produced only 44 singles. Two other diploids, offspring of Slenders that were offspring of Unstable, also gave about 25 percent doubles (56.F21 and F22). It is indicated that all these diploids descended from Unstable lacked the pollen lethal, and that in the second generation one was homozygous single—*SL/SL*.

The other single-flowered variants from Unstable parents (Tables 2 and 3) showed slenderness, and the ones tested usually gave progenies resembling those of ordinary (stable) Slender. Such progenies were given by eight parents tested at Guadalupe. One gave about 25 percent doubles, indicating that *S* was in a whole chromosome without *l*. Table 5, with exception of the last line, reports progeny tests of two generations of single Slender extracted from Unstable. These four parents appeared to behave like *sL/sL*(*Sl*) with respect to both plant char-

TABLE 5

Progenies of single trisomic Slender extracted from trisomic Unstable (Riverside)

Progeny number	Parent		Progeny						
	Generation of Slender	Origin	No. of seeds sown	No. of plants	Percent double	Diploid		Slender	
						Sg	Db	Sg	Db
56.2	1	Guad. 1955	30	27	48	2	12	12	1
56.3	1	Guad. 1955	30	15	47	1	7	7*	0
56.F12	2	56.2–16	50	41	56	3	22	15	1
56.F13	2	56.3–5	50	22	77	0	17	4†	0
56.F11	1	55.1–58	50	43	74	1	32	10‡	0

* One plant, by somatic characters, tetrasomic Slender.
† One plant with mixed flowers. This could have been a result of chromosome reversion or of cross-pollination from an Unstable plant.
‡ Some of these plants possibly diploid. (See text).

acters and ratios (except for one progeny plant noted in a footnote). *S* was probably in the fragment chromosome in all 13 of the parents that seemed to be Slender, except the one giving the 3:1 ratio. Absence of *l* in this plant, and in the tested single-flowered diploids derived from Unstable either directly or through Slenders of Table 5, may have resulted from the crossing over that placed *S* in a whole chromosome. It is possible, however, that *l* may not have been regularly present in the Unstable parents concerned and in the Slenders derived from them. Because of the close linkage, crossover diploid singles, *S/s*, from *sL/sL/(Sl)* Slender parentage, such as those of Table 1, progeny 1, are expected to have *l*, though adequate genetic evidence is not available. Parent 55.1–58 appeared substantially Slender, with some suggestions of diploid characteristics, but was completely single and fully fertile. The trisomic progeny of 55.1–58 differed in having reduced Slender characters, so that the distinction between Slender and diploid in its progeny (56.F11) was usually dubious in well-developed plants.

All completely single-flowered plants described as Slender trisomics, which have been derived from Unstable ancestry and studied cytologically, were found to differ from the usual Slender type in either size or form of the extra chromosome. In trisomic plants of 56.F11 the extra chromosome was smaller than that of ordinary Slender; in those of 56.F12 it was decidedly larger, nearly as large as a whole chromosome, and was clearly rod-shaped. In plant 55.1–66 (Figure 1), the extra chromosome was also larger than in typical Slender; it was horseshoe-shaped, and, at pachytene, was seen to be paired with both homologues. In 55.1–58 the extra chromosomes found in p.m.c. were often clearly rod-shaped and a large number were very small.

Since in 55.F11 leaf slenderness was so poorly developed that it was difficult to distinguish trisomic from diploid plants, whereas in 56.F12 they were easily separated, it seems fair to conclude that the extra chromosome of progeny 56.F12 contained more genes influencing slenderness than were present in 56.F11.

SUMMARY

1. The Unstable type of *Matthiola incana* originated from a seed of the usually somatically stable trisomic Slender, which has a rod-shaped extra chromosome approximately half the size of its normal homologue. Unstable is variegated for single and double flowers and for slender and normal leaves. The Unstable line has been maintained for four generations. Loss of a ring chromosome in mitosis is usually responsible for the variegation observed in Unstable.

2. The genetic behavior of Unstable is basically similar to that of Slender, both usually giving a majority of diploid double plants, a minority of parent type trisomics, and crossover types. Selfed, Unstable plants usually gave high or very high proportions of completely double offspring. The extra chromosome of the gamete received from Slender by the original Unstable variant, carried *S*, the gene for single flowers, and possibly also the linked pollen lethal *l* which is characteristic of the singles (*Sl/sL*) of ordinary diploid double-producing

Matthiola varieties. In the resulting Unstable plant, the two homologous whole chromosomes both carried *s* (doubleness). Occasional single-flowered diploids were produced by Unstable parents. The ones tested gave ratios indicating absence of *l* (formula, *SL/sL*). The original ring may have lacked *l* or may have lost it by breakage or by crossing over.

3. Breakage of the ring occurs because it occasionally becomes dicentric in mitosis. As a result, the progeny of Unstable plants may receive a small, medium, or large ring, a rod, or a V-shaped extra chromosome.

4. The progeny of a plant with a large ring in many p.m.c. included a great variety of Unstable plants. A plant that transmitted medium-sized rings to most of its progeny had much more uniform Unstable offspring.

5. Although most of the plants having a rod- or V-shaped extra chromosome were somatically stable, instability was, in one progeny apparently caused by occasional loss of a small rod. Why some rods were stable and others tended to be lost is not known.

6. None of the florally stable slender-leaved trisomics derived from Unstable ancestry and examined cytologically had exactly the same type of extra chromosome as the usual Slender trisomic.

7. The evidence from chromosome breakage gives some indication of the position of the gene *S* and of the gene or genes influencing slenderness. *S* was present in the smallest fragment that was found to be transmitted, and plants with this fragment had nearly normal leaves. Slenderness is thus shown to be chiefly determined by a portion of the extra chromosome distal to *S*.

ACKNOWLEDGMENTS

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CONTENTS OF PRECEDING ISSUES

JULY, 1959, PART 2

New Policies for Genetics.

STADLER, DAVID R., Gene conversion of cysteine mutants in Neurospora.

CHAO, CHUAN YING, Heterotic effects of a chromosomal segment in maize.

BONNIER, GERT, ULLA B. JONSSON, and CLAES RAMEL, Experiments on the effects of homozygosity and heterozygosity on the rate of development in *Drosophila melanogaster*.

PRICE, SAM, Chiasma terminalization in a structural heterozygote in Secale.

MOHAMED, ALY H., Inheritance of quantitative characters in *Zea mays*. I. Estimation of the number of genes controlling the time of maturity.

VAN SCHAIK, NANCY WORNER, and R. ALEXANDER BRINK, Transpositions of modulator, a component of the variegated pericarp allele in maize.

YAMAMOTO, TOKI-O, A further study on induction of functional sex reversal in genotypic males of the medaka (*Oryzias latipes*) and progenies of sex reversals.

SEPTEMBER, 1959, PART 1

New Policies for Genetics.

VETUKHIV, M. O., and J. A. BEARDMORE, Effect of environment upon the manifestation of heterosis and homeostasis in *Drosophila pseudoobscura*.

LEFEVRE, G., JR., and M. M. GREEN, Reverse mutation studies on the forked locus in *Drosophila melanogaster*.

ADAMS, M. W., and D. B. SHANK, The relationship of heterozygosity to homeostasis in maize hybrids.

HAGEN, CHARLES W., JR., Influence of genes controlling flower color on relative quantities of anthocyanins and flavonols in petals of *Impatiens balsamina*.

BENNETT, DOROTHEA, L. C. DUNN, and SUSAN BADENHAUSEN, A second group of similar lethals in populations of wild house mice.

PREER, JOHN R., JR., Studies on the immobilization antigens of Paramecium. IV. Properties of the different antigens.

KIKUDOME, GARY Y., Studies on the phenomenon of preferential segregation in maize.

HANSON, W. D., Early generation analysis of lengths of heterozygous chromosome segments around a locus held heterozygous with backcrossing or selfing.

HANSON, W. D., Theoretical distribution of the initial linkage block lengths intact in the gametes of a population intermated for *n* generations.

MITCHELL, MARY B., Detailed analysis of a Neurospora cross.

HANSON, W. D., The breakup of initial linkage blocks under selected mating systems.

SEPTEMBER, 1959, PART 2

New Policies for Genetics.

ROBERTSON, FORBES W., Studies in quantitative inheritance. XII. Cell size and number in relation to genetic and environmental variation of body size in Drosophila.

CULP, THOMAS W., Inheritance and association of oil and protein content and seed coat type in sesame, *Sesamum indicum* L.

GRELL, RHODA F., The Dubinin effect and the Y chromosome.

HINTON, CLAUDE W., A cytological study of u^{wc} chromosome instability in cleavage mitoses of *Drosophila melanogaster*.

TSUNEWAKI, KOICHIRO, and E. G. HEYNE, Radiological study of wheat monosomics. I. Differential sensitivity of monosomic X and the disomic to X-irradiation.

TSUNEWAKI, KOICHIRO, and E. G. HEYNE, Radiological study of wheat monosomics. II. Differential sensitivity of 16 monosomics and the disomic to a single dosage of X-irradiation.

BRILES, C. O., W. H. MCGIBBON, and M. R. IRWIN, Additional alleles affecting red blood cell antigens in the chicken.

IVES, P. T., The relationship between radiation dose and dominant visible mutation rate in *Drosophila melanogaster*.

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